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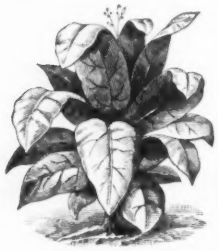

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PROPERTIES OF THE POTATO YELLOW-DWARF VIRUS[1]

L. M. Black

(Accepted for publication July 22, 1938)

INTRODUCTION

The potato yellow-dwarf virus, carried by the clover leaf hopper, *Aceratagallia sanguinolenta* Prov. (1, 2), is difficult to transmit mechanically. Muncie (9) failed to obtain infections by injection of juice from diseased leaves into healthy tubers. The writer, in preliminary experiments with the carborundum (11) and pin-puncture methods, succeeded in transmitting the virus to only an occasional plant. Further investigation of the problem has led to the discovery of a host (*Nicotiana rustica* L.) to which the virus can be transmitted with ease. The use of this species has made it possible to determine readily some properties of the virus that otherwise would have been extremely difficult or impossible to study. The following paper is an expanded account of results briefly reported elsewhere (3).

NEW HOSTS OF THE VIRUS

In the course of the investigation it was found that the yellow-dwarf virus could be transmitted to a number of plants not hitherto demonstrated to be susceptible. The new hosts listed in table 1 were inoculated by either infective leaf hoppers or by grafting. In every case the virus was transferred from the new host back to a plant upon which it produced known distinctive symptoms. At first, Green Mountain potatoes (*Solanum tuberosum* L.) were used for this purpose. Later it was found that the virus had a shorter incubation period in *Nicotiana glutinosa* L. and the latter was substituted as a test plant.

SEPARATION OF THE YELLOW-DWARF VIRUS FROM THE X-VIRUS

When potato plants were employed as a source of yellow-dwarf virus for the inoculation of other plants, symptoms were sometimes attributable, in part at least, to the X-virus, which is usually present in commercial potato varieties. A pure source of yellow-dwarf virus was indispensable for experimental purposes. In preliminary experiments it had been found that the two viruses, when introduced into *Nicotiana glutinosa* by scions from infected potatoes, produced a severe disease. To obtain the yellow-dwarf virus free from the X-virus, *N. glutinosa* plants were inoculated by means of infective leaf hoppers taken from clover. Some of the plants developed pronounced and distinctive symptoms. These plants were shown to contain the yellow-dwarf virus (Table 1) and to be free of the X-virus. Their juice failed to infect 100 Ruby King pepper plants, whereas control

[1] This study was conducted at The Rockefeller Institute for Medical Research, Princeton, N. J., during the tenure of a National Research Council Fellowship in the Biological Sciences.

TABLE 1.—*Transmission of the potato yellow-dwarf virus from new hosts to suitable test plants*

New host	Test plant to which virus transmitted	Transmitted by means of	Infections on[a]		Remarks on new host
			Test plants	Control plants	
Nicotiana glutinosa L.	*Solanum tuberosum* L. var. Green Mountain	Grafts	12/20	0/20	Distinctive symptoms
Nicotiana langsdorffii Weinm.	"	"	5/5	0/5	
Nicotiana sylvestris Spegaz. and Comes	"	"	7/10	0/10	
Callistephus chinensis Nees. var. Semple's Shell Pink	"	*Aceratagallia sanguinolenta*	7/8	0/8	Very mild symptoms
Trifolium incarnatum L.	"	"	5/5	0/5	Severe symptoms in acute stage
Vicia faba L.	"	"	8/10	0/10	
Nicotiana rustica L. (inoculated by rubbing)	"	"	2/5	0/5	
Nicotiana rustica L. (inoculated by grafts)	"	Grafts	2/5	0/5	
	Nicotiana glutinosa L.	"	7/12	0/12	
Nicotiana glauca R. Grah.	"	"	8/10	0/10	Leaves abscissed
Nicotiana paniculata L.	"	"	9/10	0/10	
Nicotiana sanderae Sander	"	"	9/10	0/10	
Nicotiana tabacum L. (Turkish)	"	"	13/20	0/20	Symptoms mild
Physalis pubescens L.	"	"	4/10	0/5	
Solanum melongena L. var. Black Beauty	"	"	10/10	0/10	

[a] The denominator represents the number of plants used, the numerator the number infected. When leaf hoppers were employed, the controls were treated exactly like the test plants except that the leaf hoppers placed on them had been fed on healthy plants of the new host instead of diseased ones.

pepper plants, inoculated with X-virus, gave 100 per cent infection. Serological tests for X-virus conducted by K. S. Chester were also negative.

The conspicuous symptoms of yellow dwarf on *Nicotiana glutinosa* (Fig. 1) suggested that this species might be a good source of inoculum. Accordingly, it was employed as a source of virus in a search for a host that would develop primary or systemic symptoms when dusted with carborundum and then rubbed with a cheesecloth pad soaked in virus-containing juice. Entirely negative results were secured in tests of 54 different species and varieties of plants. In tests with an unidentified variety of *N. rustica* L.,[2] however, yellow primary lesions (Fig. 2) developed 7–9 days after inoculation. These enlarged from their original size of about 1 mm. in diameter to a final size of about 10 mm. in diameter.

[2] An unidentified variety herein referred to as *Nicotiana rustica*.

FIG. 1. Characteristic systemic symptoms on *Nicotiana glutinosa* invaded by the potato yellow-dwarf virus. Photograph by J. A. Carlile.

MECHANICAL TRANSMISSION IN NICOTIANA RUSTICA

When the virus was transferred from *Nicotiana rustica* to *N. rustica*, much larger numbers of primary lesions were obtained than when *N. glutinosa* was used as a source. As many as 1350 lesions were counted on a single half-leaf. Sometimes a leaf bore so many lesions that it turned completely yellow a few days after the spots first appeared. Moreover, primary lesions can be obtained on *N. rustica* by rubbing the leaves without the use of carborundum. The 5 other varieties of *N. rustica* (English, Iowa, *jamaicensis, pumila,* and Winnebago) that have been tested, all developed primary lesions as a result of inoculation with yellow-dwarf virus. It is also interesting that *N. paniculata* L. and *N. undulata* Ruiz. and Pav. (5), the descendants of the probable parental species of *N. rustica,* both develop primary lesions when inoculated with the yellow-dwarf virus.

Since the work of Holmes (6), several viruses have been described as causing primary lesions on certain hosts. In the present case the growth of the lesions over a period of 2 or 3 weeks after their appearance and the subsequent development of systemic symptoms of yellow dwarf in the new growth leave little doubt as to the agent responsible for the spots. The virus from such plants has been returned to Green Mountain potatoes both

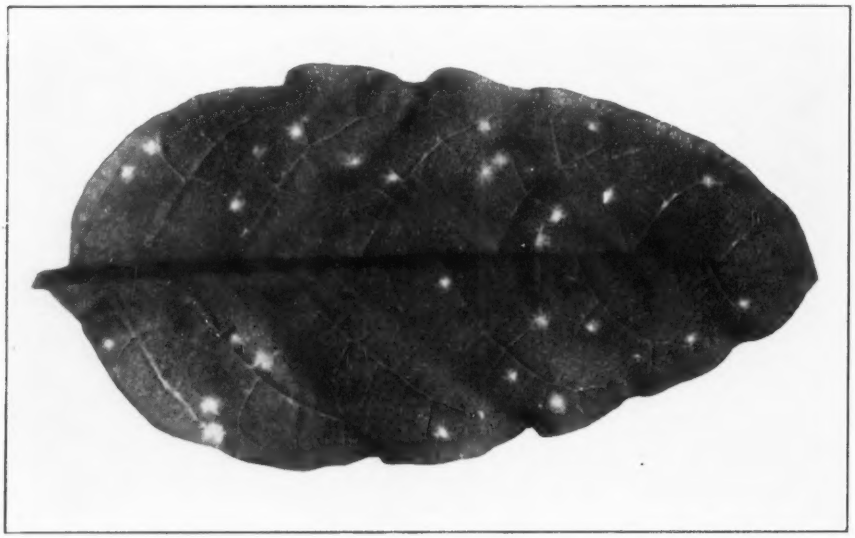

FIG. 2. Primary lesions produced by the potato yellow-dwarf virus on a leaf of *Nicotiana rustica*. Photograph by J. A. Carlile.

by grafting and by insects (Table 1), and has resulted in typical yellow dwarf.

MECHANICAL TRANSMISSION TO OTHER HOSTS

The carborundum method of transmitting the potato yellow-dwarf virus on *Nicotiana rustica* was compared with that on potato (*Solanum tuberosum* var. *Green Mountain*), *N. glutinosa*, Turkish tobacco (*N. tabacum* L.), *Hyoscyamus niger* L., and crimson clover (*Trifolium incarnatum* L.). In the first part of the experiment, juice from diseased plants of the host in question was used to inoculate healthy plants of the same species and also to inoculate *N. rustica*. From the results (Table 2), it is apparent that

TABLE 2.—*Comparison of Nicotiana rustica with other species as test plants for potato yellow-dwarf virus*

Source of the virus	Inoculation of the same species		Inoculation of *N. rustica*	
	Number of plants inoculated	Number infected	Number of half-leaves inoculated	Number of primary lesions
Solanum tuberosum L. var. Green Mountain	11	0	5	281
Nitotiana glutinosa L.	10	2	32	345
Nicotiana tabacum L. var. Turkish	20	1	5	13
Hyoscyamus niger L.	10	0	5	26
Trifolium incarnatum L.	26	0	9	482

N. rustica proved more susceptible to inoculation by the juices of these plants than did the source plants themselves. In the second part of the

experiment, juice from diseased *N. rustica* plants was used to inoculate the other host species. The results (Table 3) show that in the 3 cases in which transmission was secured the *N. rustica* juice was superior to the juice from the specific host plant in producing infections. In the case of *N. glutinosa,* apparently the most susceptible of the 5 species tested, about 5 primary lesions were produced on each inoculated leaf when *N. rustica* was used as the source of the virus. No primary lesions were observed when this species was inoculated with juice from diseased plants of the same species. These results are probably attributable to the fact that the virus reached a higher concentration in *N. rustica* than in the other species.

TABLE 3.—*Infections obtained in different species when inoculated with yellow-dwarf virus from Nicotiana rustica and other hosts*

Species inoculated	Source of inoculum			
	The same species		*Nicotiana rustica*	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected
Solanum tuberosum L. var. Green Mountain	11	0	11	0
Nicotiana glutinosa L.	10	2	19	19
Nicotiana tabacum L. var. Turkish	20	1	20	10
Hyoscyamus niger L.	10	0	10	10
Trifolium incarnatum L.	26	0	15	0

Extensive experiments were undertaken in an attempt to find a successful means of mechanical transmission from potato to potato and from clover to clover. Various methods of transmission from diseased to healthy Green Mountain potatoes were tried. Using carborundum of various grades, juice from yellowing leaves of plants in the acute stage of the disease was rubbed on the upper and lower surfaces of the leaves of young healthy plants. It seemed that transmission was difficult because of the possible rapid inactivation of the virus by oxidation. Accordingly, potassium cyanide, which inactivates aerobic oxidases and peroxidases, and sodium sulphite, a reducing agent, were tested separately for a possible beneficial action. Some inoculations were made in an atmosphere of carbon dioxide. None of these methods increased the number of infections obtained. In other experiments pins of various diameters were thrust through diseased leaves or into juice from such leaves and then into terminal buds, axilliary buds, stem tips, leaves, the eyes of the tubers, and the vascular ring of small seed pieces. Juice alone and juice mixed with carborundum were injected with a hypodermic needle into the stems and tubers. Carborundum and juice were rubbed into the freshly exposed surfaces of the split stems of young plants. The plants were observed from time to time for 4–6 weeks after inoculation. Of 980 inoculated plants surviving these treatments, 104 (10.6 per cent) developed yellow dwarf, while out of 179 noninoculated control plants none developed the disease. Most of the inoculation methods could be classified as modifications of

either the pin-puncture or the carborundum method. Of 237 plants inoculated by some modification of the carborundum method, only 6 (2.5 per cent) became diseased. On the other hand, of 670 plants inoculated by some modification of the pin-puncture method, 92 (13.7 per cent) became diseased. The best transmission obtained in any experiment was only 50 per cent. This was secured by placing juice from leaves in the acute stage of the disease around the eye of an unsprouted seed piece and making about 100 punctures with ordinary pins through the juice into the flesh of the tuber.

The symptoms of potato yellow dwarf on crimson clover are quite characteristic. Infected plants first show a pronounced clearing of the veins, which is soon followed by yellowing of the leaves. The plant is considerably dwarfed and may die. However, if it survives the acute stage of the disease, it gradually recovers and eventually appears to be healthy. Because crimson clover has proved to be the most favorable plant on which to study the insect transmission of the virus, the possibility of mechanical transmission to this host was investigated further. In the first experiment (Table 4), juice from diseased crimson clover plants in the acute stage was rubbed over the leaves of 866 plants, using carborundum as an abrasive. In addition, 98 plants were similarly inoculated with juice from infected *Nicotiana rustica* plants. The plants were 2–6 weeks old and were distributed in 5 groups, according to age. Except for the plants in the first age group, which had only one leaf at the time of inoculation, two of the compound leaves on each plant were inoculated by rubbing them 3 times. Six weeks later none of the plants showed definite symptoms of yellow dwarf. Thirty plants that appeared as though they might have early symptoms were held an additional 6 weeks. Only one of these subsequently developed yellow dwarf. This plant may have been accidently inoculated by insects, but this is considered improbable. Juice from each yellow-dwarf crimson-clover plant used as a source of virus was tested on a small interveinal area of an *N. rustica* leaf (an area about 1/10 of the leaf surface). On the average, the 36 plants tested produced 44 lesions per interveinal area, indicating that the failure to transmit was not because of any lack of virus. As shown in table 3, the same method of inoculation was successful in transmitting pea virus 2 (10) and red-clover mosaic virus (15). Comparable plants also were readily infected with yellow-dwarf virus by means of insects. The failure of the carborundum method was confirmed in a second experiment in which 158 inoculated plants were observed for 12 weeks after inoculation. In an accompanying experiment (No. 3), the infectivity of the inoculum was demonstrated by the inoculation of an additional 47 plants with 50 pin punctures through juice from diseased clover into the crown of the plant. Ten plants developed yellow dwarf, while none of the 47 control plants became diseased. It is interesting that the carborundum method, so successful for the transmission of the virus to *N. rustica*, should be so inefficient in transmitting it to potatoes and to clover.

TABLE 4.—*Transmission of 3 viruses from clover to clover*

Experiment	Virus	Source of virus	Method of inoculation	Age of plants in weeks	Leaves inoculated per plant	Plants inoculated	Plants infected	Incubation period in weeks
1	Potato yellow dwarf	Crimson clover	Carborundum	2	1	155	0	.....
	"	"	"	3	2	182	0	.....
	"	"	"	4	2	190	0	.....
	"	"	"	5	2	185	0	.....
	"	"	"	6	2	154	1	7
	"	*Nicotiana rustica*	"	2–6	2	98	0	.....
	Pea virus 2	Crimson clover	"	2–6	2	25	21	1–2
	Red clover mosaic	"	"	2–6	2	25	24	1–2
	Potato yellow dwarf	"	Insect	2–6	.....	10	9	3–6
2	Potato yellow dwarf	"	Carborundum	6	7	158	0	.....
	"	.....	Noninoculated	6	.....	158	0	.....
3	Potato yellow dwarf	"	Pin puncture	6	.....	47	10	3–5
	"	.....	Noninoculated	6	.....	47	0	.....

PROPERTIES OF THE VIRUS

The primary-lesion reaction on *Nicotiana rustica* was applied in a study of some of the properties of the virus. Experience has shown that much better results are obtained with this reaction during the winter months than during the summer. It was soon discovered that the age of the *N. rustica* plant and the age of the leaf were important factors in determining the number of lesions. Plants 7–9 weeks old with stems that had not yet rapidly elongated appeared to be most suitable. On such plants the youngest expanded leaf and the next oldest leaf were selected for inoculation. The growing point was removed, the leaves were dusted with No. 320 carborundum, rubbed three times with a cheesecloth pad soaked in inoculum, and washed with tap water. The plants were grown in a greenhouse held at 80° F. or higher, and the primary lesions were counted after about 2 weeks. A sample of juice was applied to just half a leaf on any one plant. Thus, 4 samples could be applied to each plant. Except for the first 2 experiments on thermal inactivation, each experiment was designed so that 4 samples to be compared were applied on every plant and in every leaf position the same number of times (14). In the experiments on dilution and thermal inactivation, where 7 samples were compared, the test plants were divided into 2 equal groups. In one group the first 4 samples were tested and in the other group the last 4 samples were tested, sample No. 4 being tested in each group as a check on the uniformity of the plants. In these cases the total number of lesions obtained for sample 4 was divided by 2 before being incorporated in the tables.

Longevity in vitro at Room Temperature.—Preliminary experiments indicated that the virus survives only a few hours in nontreated juice held at room temperature (23–27° C.). In 2 experiments (Table 5), in which the activity

TABLE 5.—*Total number of primary lesions produced on half-leaves of Nicotiana rustica after holding juice from diseased N. rustica plants at room temperature for various intervals*

Experiment	Number of half-leaves	Interval					
		0 hours	1 hour	2½ hours	4 hours	8 hours	12 hours
1	20	4859	2592	6	2	.....	.....
2	20	2055	209	18	0	.....	.....
3	16	10236	.....	.....	2558	154	5
4	16	3732	.....	.....	35	0	0

of extracts of yellow-dwarf *Nicotiana rustica* leaves was tested 1, 2½, and 4 hours after extraction, the results indicated that the virus was almost completely inactivated after 2½ hours. However, it became apparent in subsequent experiments designed for other purposes that this was not always so, and in 1 of 2 later experiments (3 and 4) it was shown that the virus survived as long as 12 hours.

An experiment was conducted to determine whether this rapid loss of

infectivity was due to the destruction of the virus or to the toxic effect of old juice upon the test plant. Juice from diseased and from healthy *Nicotiana rustica* plants, respectively, was held at room temperature for either 13 hours or for 0 hours. Four solutions were then prepared as follows and tested immediately on *N. rustica*.

Solution 1. 9 cc. of juice from diseased plants held 13 hours + 1 cc. of such juice held 0 hours.

Solution 2. 9 cc. of juice from healthy plants held 0 hours + 1 cc. of juice from diseased plants held 0 hours.

Solution 3. 10 cc. of juice from healthy plants held 0 hours.

Solution 4. 9 cc. of juice from diseased plants held 13 hours + 1 cc. of juice from healthy plants held 0 hours.

When tested on 16 half-leaves, solutions 1 and 2 produced lesions (120 and 234, respectively), but solutions 3 and 4 did not. This shows that the loss of infectivity within 13 hours is not due to the formation of a substance that interferes with the entrance of the virus into the plant, but is due to destruction of the virus. The experiments on longevity indicate that this destruction is very rapid.

Longevity of Virus in Dried Leaves.—Leaves from yellow-dwarf *Nicotiana rustica* plants in the acute stage of the disease were allowed to dry in the laboratory for 1, 2, 4, 12, and 16 weeks. The various samples were then ground up separately in a small volume of 0.1 M neutral phosphate buffer. Each extract so obtained was tested on 10 half-leaves of *N. rustica*. None gave any lesions.

Dilution.—Dilution experiments have been performed using both phosphate buffer at pH 7.0 and healthy *Nicotiana rustica* juice as the diluents. Dilutions up to 10^{-5} were tested, and one sample of the diluent alone was included in each experiment as a control. None of these control samples produced any lesions and they are omitted from table 6, which gives the results of the dilution tests. In 3 experiments no lesions were obtained at dilutions higher than 10^{-3}. In one experiment, 1 infection was obtained at a dilution of 10^{-5}. Because of the large number of primary lesions obtained in some other experiments where undiluted juice was employed, it would not be surprising if infections were obtained at even higher dilutions under the most favorable circumstances for inoculation.

TABLE 6.—*Primary lesions produced on half-leaves of Nicotiana rustica with various dilutions of yellow-dwarf N. rustica juice*

Experiment	Number of half-leaves used	Diluent	Total number of lesions produced at a dilution of					
			10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	10^{0}
1	8	Healthy juice	0	0	9	85	474	634
2	4	Buffer	.....	0	1	8	141	871
3	8	"	1	10	118	996	1421	1952
4	8	"	0	0	2	90	693	100

Thermal Inactivation.—Because of the rapid inactivation of the potato yellow-dwarf virus *in vitro* at room temperature, a single stock solution of juice could not be employed as a source of samples for the various temperature treatments in an experiment. Instead, a large number of leaves were cut up into small pieces about 6 square centimeters in area and the pieces thoroughly mixed. The chopped leaves were held under moist paper towels. A small sample was withdrawn for each temperature determination, ground in a mortar, and about 5 cc. of juice squeezed out through 2 layers of gauze. Samples of this juice were pipetted into small test tubes (7 x 1 cm.) and immersed for 10 minutes in a water bath at the desired temperature. Upon withdrawal, the tubes were cooled rapidly by placing them in cold water, and the treated juice was applied immediately to half-leaves of *Nicotiana rustica*. In experiments 1 and 2 (Table 7) only 1 leaf was used on each test plant, half of the leaf being used to test the sample and the other half being left noninoculated as a check. In experiments 3 and 4, the samples were compared in the manner previously described. The results indicate a thermal inactivation temperature of about 50° C.

TABLE 7.—*Number of primary lesions produced on half-leaves of Nicotiana rustica inoculated with samples of yellow-dwarf virus heated for 10 minutes at various temperatures*

Experiment	Half-leaves used	Total number of lesions produced after inoculation with juice heated to the following temperatures (°C.)									
		56°	54°	52°	50°	49°	48°	47°	46°	45°	25°
1	2	.....	.....	.....	3	.....	7	10	29	113	.....
2	2	0	0	0	0	.....	8	.....	0	.....	1524
3	8	0	0	0	0	.....	0	.....	2031	.....	4923
4	8	.....	.....	.....	0	13	25	28	109	133	2532

Filtration.—Two hundred grams of yellow-dwarf *Nicotiana rustica* leaves were ground with 8 gm. of K_2HPO_4 in a large mortar. The juice was squeezed through 4 layers of cheesecloth and filtered through celite (Standard Super-cel). Two cc. of a 48-hour culture of *Phytomonas stewarti* (E.F.S.) Bergey *et al.* were added to the celite filtrate and the mixture was then passed through a Berkefeld W filter under a pressure of 1 atmosphere. A 1-cc. sample of the celite filtrate to which the bacteria had been added was placed in each of 2 test tubes of nutrient broth. The Berkefeld filtrate was similarly tested in 2 more tubes. Samples of the juice, celite filtrate, and Berkefeld filtrate showed a pH of 7.1 when tested by the glass electrode method. The 3 solutions, undiluted and diluted 1:40 in phosphate buffer at pH 7.1, were tested on *N. rustica* half-leaves. Control inoculations were made with the sterile buffer. So many lesions were obtained in the case of the undiluted samples of juice and the 2 filtrates that lesions were counted only on a disc 2 cm. in diameter cut from the middle of each half-leaf. In the other cases all lesions were counted. The test tubes of broth to which samples of the Berkefeld filtrate had been added did not show any growth after 2

weeks, whereas the test tubes containing the celite filtrate developed a dense growth within 2 days. These results, demonstrating the filterable nature of the virus (Table 8), were confirmed in a second experiment.

TABLE 8.—*Total number of primary lesions produced on half-leaves of Nicotiana rustica after various filtration treatments of juice from diseased N. rustica plants*

Experiment	Dilution used	Half-leaves inoculated	Total number of lesions on half-leaves inoculated with			
			Berkefeld filtrate	Celite filtrate	Unfiltered juice	Phosphate buffer
1	None	8	221[a]	442[a]	540[a]	0
	1: 40	8	65	52	11	0
2	None	4	1203	1306	1200	0

[a] Lesions were so numerous on these half-leaves that they were counted only on a small disc cut from the center of each half-leaf with a cork-borer.

DISCUSSION

The potato yellow-dwarf virus is carried by the clover leaf hopper (1, 2). In experiments previously reported (2), the writer failed to obtain transmission by the common aphids occurring on potatoes and considered that the ability of aphids to transmit the virus (8, 9) was doubtful. With the exception of this virus, Johnson and Hoggan (7) classify all the leaf-hopper-borne viruses in one group characterized in part by the difficulty with which they are transmitted mechanically. In the group, the only viruses that have been so transmitted are those of curly top of beets, with which Dana (4) obtained 50 per cent transmission from plant to plant by pin puncture, and the virus of maize streak, which Storey (12) transmitted from insect to insect. Oddly enough, Storey (13) has been unable to transmit this virus mechanically from plant to plant.

As shown in the present study, the mechanical transmission of the potato yellow-dwarf virus to potato and to crimson clover is comparable with that of the curly-top virus on beets, as reported by Dana. On both these hosts pin punctures into a region where the vascular bundles were aggregated was superior to the carborundum method of leaf inoculation. This may indicate that on these plants the main factor for successful inoculation is the introduction of the virus into the phloem. On *Nicotiana rustica*, however, the success of the carborundum method in producing primary lesions suggests that in this plant the entrance of the virus into the phloem is not necessary and indicates the importance of the host plant in the mechanical transfer of the virus.

Where the properties of other leaf-hopper-borne viruses have been investigated, the technique has involved the laborious process of first introducing the virus into the insect vector and then testing the insect on susceptible plants. The greater ease with which the yellow-dwarf virus has been studied on *Nicotiana rustica* furnishes a striking contrast. Unless this virus is unique among those carried by leaf hoppers, one may expect that some

of the other viruses in this group will prove to be readily transmissible mechanically to certain of their hosts.

SUMMARY

Several new hosts of the potato yellow-dwarf virus are reported.

The virus was shown to produce primary lesions upon *Nicotiana rustica* when leaves of a suitable age were inoculated by the carborundum method. This host has proved important in investigating properties of the virus.

Mechanical transmission of the potato yellow-dwarf virus from potato to potato or clover to clover proved difficult. The carborundum method was inefficient in effecting transmission on these hosts. The best method of mechanical inoculation on potato consisted of pin punctures into the tuber near an eye. On clover, transmission was secured by pin punctures into the crown.

The primary-lesion reaction was applied to the study of some properties of the virus. The virus in juice of *Nicotiana rustica* was destroyed at room temperature in from $2\frac{1}{2}$ to 12 hours. It did not remain active in dried leaves. The virus could be recovered consistently from yellow-dwarf *N. rustica* juice diluted 10^{-3} with phosphate buffer or healthy juice, and in one experiment infections were obtained with a dilution of 10^{-5}. Its thermal inactivation point was found to be about 50° C. The virus passed through a Berkefeld W filter.

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STUDIES ON POLYPORUS ABIETINUS. II. THE UTILIZATION OF CELLULOSE AND LIGNIN BY THE FUNGUS[1]

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Cellulose and lignin, the two most important constituents of wood, comprise approximately 50 per cent and 30 per cent, respectively, of the dry weight of coniferous woods. Materials present in this magnitude, therefore, must play important rôles in the metabolism of wood-destroying fungi. Xylophilous fungi, consequently, have been classified into 2 groups, the brown-rot fungi and the white-rot fungi. This classification is based upon the supposition that certain fungi destroy only cellulose, leaving the brown lignin, and that others destroy only lignin, leaving the white cellulose. Falck and Haag (3) proposed the term "destruction fungi" for the brown-rot group, and "corrosion fungi" for the white-rot group. Campbell (2), however, demonstrated that wood decayed by single species is lower both in cellulose and in lignin than undecayed wood, and, consequently, that certain fungi are able to destroy both cellulose and lignin. Among the fungi regarded by Campbell as destroying both of these constituents simultaneously, is *Polyporus abietinus* (Dicks.) Fries. This fungus, however, had previously been classified by Thaysen and Bunker (6) as a brown-rot fungus and by Hubert (5) as a white-rot fungus. The purpose of the study here reported was to test the ability of *P. abietinus* to utilize cellulose or lignin, singly as a source of nutrition. If it is able to utilize both materials in culture it may be assumed that, when growing in wood, it could also destroy both materials. If, however, it is able to utilize only one or the other of the constituents then it could not be anticipated that both serve as food under natural conditions.

Methods

In testing the ability of *Polyporus abietinus* to utilize either cellulose or lignin as a source of nutrition, the fungus was grown on media containing either cellulose or lignin as the only organic nutritional material. For this purpose a mineral stock agar composed of the following ingredients was prepared:

Distilled water, 1,000.0 g.; $MgSO_4$, 0.5 g.; K_2HPO_4, 1.0 g.; KCl, 0.5 g.; $FeSO_4$, 0.1 g.; agar-agar, 20.0 g.

This stock agar, alone, did not support the growth of the fungus. To the stock medium, therefore, 2.5 per cent cellulose gel was added. This cellulose gel was prepared from filter paper by use of Schweitzer's reagent. A lignin medium was prepared by adding 2.5 per cent isolated lignin to the stock medium. This lignin was obtained from pine wood by use of the sulphuric

[1] A portion of a thesis submitted to the Graduate Faculty of Duke University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] The author herewith gratefully acknowledges his indebtedness to Dr. Frederick A. Wolf under whose guidance this work was conducted.

acid method of isolation. A portion of the cellulose medium and a portion of the lignin medium were further enriched by the addition of 1 per cent ammonium nitrate.

Poured plates of these media were inoculated with a vigorous culture of *Polyporus abietinus* and were incubated at 30° C. for the periods listed in table 1. At the end of this period the radial growth of the colonies was measured.

Bavendamm (1) advocated the use of another method for testing the ability of a fungus to utilize lignin. In this method the fungus is grown on a medium containing tannic acid. If a dark brown halo is formed, then it is assumed that the fungus is able to digest lignin. According to Bavendamm (1) lignin and tannic acid are closely related substances. The decomposition of tannic acid, shown by the formation of the brown decomposition products, is evidence that the similar substance, lignin, also can be destroyed. In the present study Bavendamm's method also was employed. For this purpose a tannic acid medium was made by adding 0.5 per cent tannic acid to the stock agar. Poured plates of this medium were inoculated with *Polyporus abietinus* and were incubated for the periods indicated in table 1.

RESULTS

The results of these cultural studies with *Polyporus abietinus* may be presented most concisely in the following table:

TABLE 1.—*Growth of Polyporus abietinus on cellulose, lignin, and tannic acid media*

Medium	No. of plates	Days growth	Average growth in millimeters	Character of growth
Stock agar and 2.5 per cent cellulose	11	9	29	fair
Stock agar and 2.5 per cent lignin	10	30	23	very sparse
Stock agar, 2.5 per cent cellulose, 1 per cent NH_4NO_3	10	9	28	fair
Stock agar, 2.5 per cent lignin, 1 per cent NH_4NO_3	11	30	30	sparse
Stock agar and 0.5 per cent tannic acid	12	9	12	good, dark brown halo

DISCUSSION

The growth of *Polyporus abietinus* on media containing either cellulose or lignin as a source of organic nutrition indicates that this fungus is able to utilize both of these components of wood. Thus the evidence obtained from cultural studies corroborates the evidence obtained by Campbell (2) for the simultaneous destruction of lignin and cellulose in wood by this fungus. It is evident, however, that cellulose *per se* is a better source of nutrition for the fungus than is lignin.

The exact nature of the lignin complex of wood is, at present, unknown.

It is fairly generally accepted, however, that it is composed largely of methoxyl, hydroxyl, acetyl, and phenolic groups. Energy would be released if these groups were acted upon by oxidizing enzymes, but no assimilable products would be furnished. According to Hawley and Wise (4), there also is evidence that pentosans are a part of the lignin complex, while others regard the pentosans as impurities resulting from the technique of isolation. The sulphuric acid employed in the isolation of the lignin for the present study should have hydrolyzed any pentosans that may have been loosely associated with the lignin. The products of this hydrolysis should have been removed in the subsequent washing of the residue. The growth of *Polyporus abietinus* on the medium containing the isolated lignin, consequently, indicates that there are materials intimately associated with the complex organic groups of the lignin that are released as sources of nutrition only after oxidizing enzymes have caused a partial breakdown of these complex organic groups. Such nutritional materials, however, are apparently present in very small amounts as evidenced by the poor growth of the fungus.

Obviously lignin isolated from wood is not identical with lignin in wood. Nevertheless, it is logical to assume that an organism capable of producing the enzymes necessary for the utilization of isolated lignin would also be able to utilize lignin in wood. *P. abietinus* grows better on the lignin medium enriched with ammonium nitrate than on the lignin medium not so enriched. It did not grow any better, however, on the cellulose medium enriched with ammonium nitrate than on the cellulose medium without the ammonium nitrate. This indicates that nitrogenous materials are of more importance in the utilization of lignin by the fungus than in the utilization of cellulose.

The formation of the brown halo on the tannic acid medium indicates that *Polyporus abietinus* forms the enzyme laccase. Gallic acid, a phenol resulting from the decomposition of the tannic acid, is oxidized by laccase with the resultant formation of dark brown pigment. The reason for assuming, as advocated by Bavendamm (1), that a wood-destroying fungus which produces a brown halo on tannic acid medium is a corrosion or white-rot fungus, and that one which does not produce the brown halo is a brown-rot fungus appears never to have been adequately explained. It seems probable that the enzyme laccase is produced by certain fungi, and that it catalyzes the oxidation of the phenolic groups of the lignin. The oxidation of these phenolic groups, therefore, constitutes a partial decomposition of the lignin complex. It should be emphasized, however, that the production of the brown halo on tannic acid medium need not be interpreted to show that the fungus utilizes lignin alone and leaves the cellulose intact. The present experiments with *P. abietinus* show that this fungus can utilize both lignin and cellulose, and therefore indicate that the Bavendamm test is not a specific means of distinguishing between the two groups of wood-destroying fungi.

SUMMARY

Polyporus abietinus utilizes either cellulose or isolated lignin as a source of nutrition. Cellulose is a better source of nutrition than is lignin.

Nitrogenous materials are of more importance in the growth of the fungus on lignin than in the growth of the fungus on cellulose.

The fungus forms a brown halo when grown on tannic acid medium, indicating the formation of the enzyme laccase. The laccase also probably catalyzes the oxidation of the phenolic groups in lignin, hence causing a partial decomposition of the lignin.

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THE INHERITANCE OF SCAB RESISTANCE IN CERTAIN CROSSES AND SELFED LINES OF POTATOES

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Common scab is widely distributed over the potato-producing areas of the world. The organism causing this disease, *Actinomyces scabies* (Thaxter) Güssow, is carried on seed potatoes and lives over from year to year in the soil. Control has been based on seed treatment. A number of seed treatments have been recommended that kill the organisms carried on the seed tubers, but no one has yet devised a method of treatment or a system of rotation that will completely eliminate scab infection arising from soil-borne organisms.

In recent years, therefore, potato breeders in the United States and elsewhere have been attempting to produce varieties resistant to the disease.

Experiments to determine whether any of the existing varieties were resistant have been made in many countries for many years. A few examples of the results will suffice to show that varieties have been known to differ in their reaction to the scab organism.

Among the early tests are those by Beckwith (1) in New York in 1887; Humphrey (5) in Massachusetts in 1890; Williams (12) in South Dakota in 1894 and 1895; and Shepperd and Ten Eyck (10) in North Dakota in 1902. In all of these experiments differences were found in the amount of scab on the tubers of the different varieties.

Stuart (11) found that although none of the 65 varieties tested were highly resistant to scab, some appeared to be less subject to the disease than others.

In tests of a large number of varieties by Schlumberger (8, 9) in Germany, a relatively high degree of resistance to scab was found in a small percentage of the varieties tested. Chief among these are Richter's Jubel, Arnica, Hindenburg, Oststarke, Robinia, Treff As, Rheingold and Ostragis.

The data at hand, covering a considerable range of varieties under widely different conditions, indicate that there are varying degrees of resistance to scab, although immunity has not yet been observed. Concerning this point, Berkner (2) says: "Absolute immunity does not appear to exist, but there are decided hereditary differences in the degrees of resistance and susceptibility."

The nature of resistance to scab has been studied by several investigators.

The fact that the most resistant American varieties possess a thick russet skin, has led some to believe that resistance is dependent upon this type of skin. Histological studies of several varieties by Lutman (7) led him to conclude that thickness of skin determines the resistance of the tubers to scab and that color is not important.

Stuart (11) pointed out the fallacy of the prevailing idea that the russet type of skin is the basis of freedom from scab and cites as an example the behavior of the Cambridge Russet, on the tubers of which scab was abundant during a period of 6 years.

In testing many seedlings, Darling, Leach, and Krantz (4) found a high degree of resistance in smooth and thin-skin seedlings, as well as in russet types. No correlation was found to exist between color of tuber and scab resistance.

Extensive studies by Longrée (6) of the skin structure of the tubers at different stages of growth showed no correlation between resistance to scab and time of periderm building, course of epiderm development, final thickness of skin, time of change of stomata to lenticels, or form, size, elevation, or depression of lenticels. Resistance was found to be associated with close packing of the "stuffing" cells of the lenticels, the degree of lenticel cambium corking, and limited ability to form wound periderm.

In the collection of seedling varieties studied by Darling, Leach and Krantz (4) the same characteristics were found associated with resistance as those reported by Longrée.

Resistance to scab is held by Wingerberg (13) to rest upon a purely physiological basis.

TESTS AT PRESQUE ISLE, MAINE

The research of the United States Department of Agriculture at Presque Isle, Maine, which was begun in 1930, has included tests of both American and foreign varieties, and progenies of a number of crossed and selfed lines. A preliminary report of this work by Clark *et al.* (3) shows that, among

the American varieties, Green Mountain, Irish Cobbler, Charles Downing, Warba, Katahdin, and Chippewa were highly susceptible to common scab and that Russet Rural, Russet Burbank, Mahr's Russet, Golden, American Giant, and an unnamed seedling, No. 44537, were intermediate in their reaction, and would be considered moderately resistant. None of the American varieties tested showed a high degree of resistance. On the other hand, the European varieties, Hindenburg, Richter's Jubel, Ackersegen, Arnica, and Hindenburg × Centifolia No. 9 were proved highly resistant. All of these introductions produced less than 1 per cent of the amount of scab found on the Green Mountain checks. None of them had enough lesions on the tubers to classify them as scabby, while the Green Mountain tubers in the check plots could not have been sold for first-class table stock.

Besides the named varieties, both American and foreign, a number of progenies have been tested during the last 4 years in an attempt to determine the genetic behavior of some of the resistant and susceptible sorts, and the possible combination of resistance to this disease with other characters of economic importance. The seedling variety used most extensively in the series of crosses presented in this paper is No. 44537. It is self-fertile, has a heavy russet skin, and is moderately resistant to scab. For these reasons, in part, it was used extensively in many of the early crosses, and, also, because its fertility made possible the production of selfed lines, crosses, and back crosses. It has, however, produced consistently low yields, and the lines obtained by selfing lack vigor; hence, other, more vigorous fertile sorts have replaced it in the recent breeding studies.

In the study of scab resistance in the various progenies the first scab data are secured from the seedlings grown from true seed. Although they are grown on nonlimed soil, a relatively severe epidemic usually prevails, so that it is possible to separate them into 2 classes, those with very little or no scab, indicating either escape or resistance, and those sufficiently scabby to clearly indicate susceptibility. The former are saved and planted in the scab test plots the following season. During the last 4 years the latter group of seedlings have been planted in soil known to have produced scabby tubers in previous years, which has received yearly applications of lime at the rate of about 1 ton per acre. Each seedling is grown in 5-hill plots, each hill alternating with a hill of Green Mountain, which is very susceptible to scab.

The method of taking the scab data has changed to some extent during the progress of the experiment, and further changes will be required before it is entirely satisfactory. In judging the reactions of the varieties and checks two criteria must be taken into consideration, the percentage of tuber surface covered with scab, and the type of pustule. Figure 1, which facilitates the reading and makes the estimates more reliable, shows tubers in 6 classes: 0, 10, 30, 50, 70, and 90 per cent of surface covered. This illustration was made by actual measurement. It is especially helpful in getting accurate readings on the Green Mountain checks, on which the reliability of the test depends. Figure 2 shows various types of scab lesions. Four types

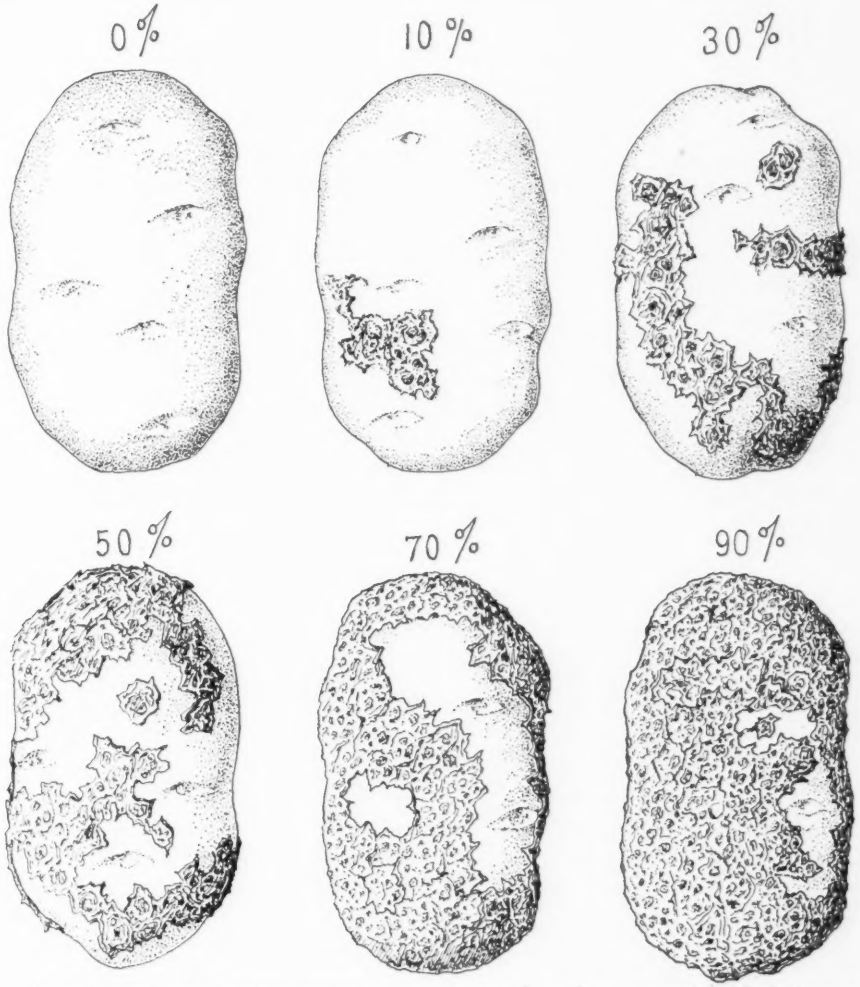

FIG. 1. Tubers showing various percentages of surface covered with lesions of common scab.

are represented, 3 pustule and one russeting type. No. 1 shows possible susceptibility in spite of the fact that only a few pustules are found on the surface, and would be considered susceptible if the Green Mountain check in the adjacent hills showed the same percentage of surface covered. On the other hand, if the tubers of the seedling variety under test showed only a few scattered pustules of type 1, and the Green Mountain check showed a very much higher percentage, the seedling variety would be considered more resistant than the Green Mountain. Types 2 and 3 without doubt indicate resistance. Type 4 is the most difficult to evaluate. While it is quite certain that scab will produce this type, other factors of environment may cause a similar condition, and there may be confusion with natural russeting conditioned by genetic factors. Because so little is known concerning this type, it has not been taken into consideration in the data here presented.

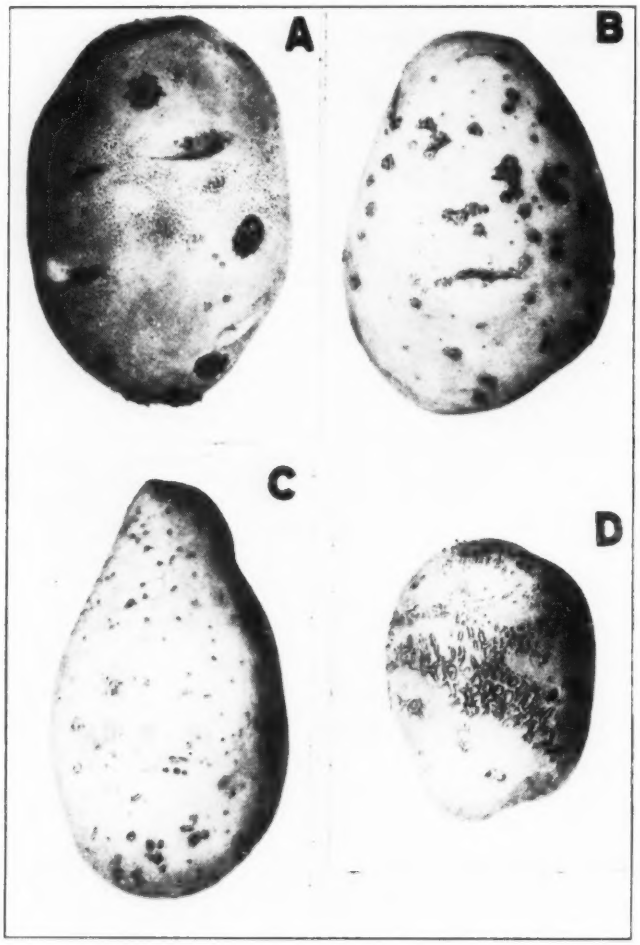

FIG. 2. Types of common scab found on potato varieties showing various degrees of resistance to the disease. A. Type 1. B. Type 2. C. Type 3. D. Type 4.

RESULTS

The progeny of a cross between 2 susceptible parents, Columbia Russet and Katahdin, were completely susceptible. The tubers of the 392 seedlings of this cross showed a high percentage of the surface covered with No. 1 pustules. In contrast to this, all of the 827 seedlings of the cross between 2 highly resistant varieties (Hindenburg × Ostragis) were as resistant as either of the parents. All of the other crosses segregated for resistance and susceptibility (Table 1). The progenies of all the crosses with at least one russet-skin parent segregated for russet and non-russet types of skin. The highly susceptible seedlings were discarded without further test. The seedlings showing little or no scab, however, were given a more severe test the following year. They were planted in soil that was known to have produced scabby tubers in previous years and to which

TABLE 1.—*Reaction to pustule types of common scab of the first-year-seedling progenies at Presque Isle, Maine*

Pedigree No.	Parents	Reaction of parents	Seedlings	Susceptible; discarded		Little or no scab; saved	
				Russet	Non-russet	Russet	Non-russet
			Number	*Number*	*Number*	*Number*	*Number*
× 146	Columbia Russet × Katahdin	Suscept. × suscept.	392	.....	392	.....	.....
× 277	Columbia Russet × S 44537	Suscept. × mod. resist.	420	61	224	111	24
× 295	Russet Rural × S 44537	Mod. resist. × mod. resist.	350	34	201	79	36
× 528	Richter's Jubel × S 44537	Resist. × mod. resist.	621	4	211	216	190
× 574	Arnica × S 44537	do	70	5	37	17	11
× 920	S 44537 × S 46422	Mod. resist. × suscept.	271	51	133	67	20
× 1037	S 45208 × S 44537	Suscept. × mod. resist.	354	68	178	58	50
× 161	Richter's Jubel × Earlaine	Resist. × suscept.	61	.....	10	.....	51
× 570 × 1002	Hindenburg × Ostragis	Resist. × resist.	827	.....	0	.....	827
× 579	Rural New Yorker × S 44537	Suscept. × mod. resist.	457	27	298	92	40
× 601	Arnica × Ostragis	Resist. × resist.	20	.....	2	.....	18
× 627	Hindenburg × Katahdin	Resist. × suscept.	900	.....	428	.....	472

TABLE 2.—*Reactions to pustule types of common scab of seedlings that showed little or no scab in 1935; grown from tubers in 1936 in limed soil with Green Mountain checks in alternate hills*

Pedigree No.	Parents	Skin character	Classes of infection						Total No. seedlings	χ^2 test for each group compared with its checks
			0	1–20	21–40	41–60	61–80	81–100		P
				Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
× 277	Columbia Russet × S 44537	Russet	...	64	33	6	3	.....	106	Less than 1 per cent
	Green Mountain check	White	...	.....	.....	19	26	61	106	
	Columbia Russet × S 44537	do	...	5	2	6	7	4	24	Approximately 25 per cent
	Green Mountain check	do	...	.....	.....	6	6	12	24	
× 295	Russet Rural × S 44537	Russet	...	59	18	.....	.....	.....	77	Less than 1 per cent
	Green Mountain check	White	...	.....	.....	24	47	6	77	
	Russet Rural × S 44537	do	...	.....	9	20	7	.....	36	Approximately 7 per cent
	Green Mountain check	do	...	.....	.....	17	18	1	36	
× 528	Richter's Jubel × S 44537	Russet	...	165	40	10	1	.....	216	Less than 1 per cent
	Green Mountain check	White	...	.....	.....	29	94	93	216	
	Richter's Jubel × S 44537	do	...	35	90	62	3	.....	190	Less than 1 per cent
	Green Mountain check	do	...	.....	.....	30	85	75	190	
× 574	Arnica × S 44537	Russet	...	10	5	2	.....	.....	17	Less than 1 per cent
	Green Mountain check	White	...	.....	.....	5	9	3	17	
	Arnica × S 44537	do	...	.....	7	3	1	.....	11	Approximately 8.0 per cent
	Green Mountain check	do	...	.....	.....	7	4	.....	11	
× 920	S 44537 × S 46422	Russet	...	50	13	3	.....	.....	66	Less than 1 per cent
	Green Mountain check	White	...	.....	.....	5	18	43	66	
	S 44537 × S 46422	do	...	.....	1	16	2	1	20	Less than 1 per cent
	Green Mountain check	do	...	.....	.....	.....	9	11	20	
×1037	S 45208 × S 44537	Russet	...	.....	36	20	1	.....	57	Less than 1 per cent
	Green Mountain check	White	...	.....	.....	19	19	19	57	
	S 45208 × S 44537	do	...	.....	2	27	16	6	51	Approximately 6 per cent
	Green Mountain check	do	...	.....	.....	11	21	19	51	
Inb. 1241	Katahdin selfed	do	...	.....	3	28	10	9	50	Approximately 1 per cent
	Green Mountain check	do	...	.....	.....	12	8	30	50	

lime had been applied each year at the rate of about 1 ton per acre. Susceptible Green Mountain was planted in alternate hills. The russet and non-russet seedlings of each cross are grouped separately in tables 2 and 3, each group being followed by its Green Mountain check. Each group was compared with its checks by the χ^2 test for goodness of fit on the basis that, so far as reaction to scab is concerned, the seedlings and their checks could both be samples of a population the parameter of which is the mean of the 2 samples. The P values are given in the tables. In every progeny the russeted segregates were more resistant than the Green Mountain variety, the differences being highly significant. The white segregates showed on the average less resistance than their russeted sibs. In crosses 277, 295, 574, and 1037 significant differences between the white segregates and their Green Mountain checks could not be demonstrated. In all the other crosses the differences between them were highly significant.

The reaction of 3 selfed lines, seedling No. 44537 selfed, Katahdin selfed, and Green Mountain naturally pollinated, is interesting. The latter is considered a selfed line, since the seed was obtained in a field of Green Mountains with no fertile varieties in close proximity. Seedling 44537 is moderately resistant to scab. It will be noted that it is one of the parents of most of the crosses reported in this paper. A selfed line of this variety segreated for russet and white skin and for resistance and susceptibility to scab. Both the russet-skin segregates and their white-skin sibs differed significantly from Green Mountain (Table 3). Katahdin is as susceptible to scab under the conditions of these tests as Green Mountain. It breeds true for white skin, yet the probabilities are less than 1 in 100 that the progeny of Katahdin, selfed, and the Green Mountain variety react alike to the pustule types of scab (Table 3). On the other hand, the progeny of Green Mountain selfed and the same number of lots of the Green Mountain variety could be drawn 90 per cent of the time from a population of which the mean of the two is the parameter.

It is evident that in these progenies russeting is associated to a high degree with resistance to the pustule types of scab. The complete data for several segregating progenies with the P values found in comparing the russet segregates with their white-skin sibs are given in table 4. In 6 crosses the probabilities are less than 1 in 100 that the russet and white-skin types belong in the same population, as far as scab resistance is concerned. That there is not a complete correlation between russeting and scab resistance is shown by the fact that in each of the crosses a number of russeted segregates were found completely susceptible to the disease. One cross, Hindenburg × Ostragis, segregated for red and white color of tubers. This whole progeny was highly resistant to scab (Table 3). It can be seen without applying the goodness-of-fit test that these two groups are almost exactly alike in their reaction to scab, and that scab resistance, therefore, is not correlated with color of tubers in this cross. If the progeny of Katahdin, selfed (Table 2), is compared with the white segregates of the

TABLE 3.—*Reactions to pustule types of common scab of seedlings that showed little or no scab in 1936; grown from tubers in 1937 in limed soil with Green Mountain checks in alternate hills*

Pedigree No.	Parentage	Skin character	Classes of infection							χ^2 test for each group compared with its checks
			0	1–20	21–40	41–60	61–80	81–100	Total	P
				Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*		
× 161	Richter's Jubel × Earlaine	White	4	23	1	1	.....	.....	29	Less than 1 per cent
	Green Mountain check	do	.....	.....	6	23	.....	.....	29	
× 579	Rural N. Y. × S44537	Russet	66	9	2	1	.....	.....	78	do
	Green Mountain check	White	.....	1	11	38	27	1	78	
	Rural N. Y. × S44537	do	1	8	13	7	1	.....	30	Approximately 2 per cent
	Green Mountain check	do	.....	.....	5	15	10	.....	30	
× 601	Arnica × Ostragis	Not russet	12	4	.....	.....	.....	.....	16	Less than 1 per cent
	Green Mountain check	do	.....	1	3	8	4	.....	16	
× 627	Hindenburg × Kat.	White	138	152	11	.....	.....	.....	301	do
	Green Mountain check	do	.....	2	80	160	44	15	301	
× 627S[a]	Hindenburg × Kat.	do	114	10	.....	.....	.....	.....	124	do
	Green Mountain check	do	.....	70	44	10	.....	.....	124	
× 570[a] and × 1002[a]	Hindenburg × Ostragis	Red	333	4	.....	.....	.....	.....	337	do
	Green Mountain check	White	.....	143	129	47	14	4	337	
	Hindenburg × Ostragis	do	343	4	.....	.....	.....	.....	347	do
	Green Mountain check	do	.....	161	116	43	15	11	347	
Inb. 1364[a]	S44537 selfed	Russet	16	10	1	.....	.....	.....	27	do
	Green Mountain check	White	.....	5	15	7	.....	.....	27	
	S44537 selfed	do	8	23	7	1	.....	.....	39	Approximately 2 per cent
	Green Mountain check	do	.....	8	12	17	2	.....	39	
Inb. 1241	Katahdin selfed	do	.....	40	45	18	1	.....	104	Less than 1 per cent
	Green Mountain check	do	.....	1	8	66	29	.....	104	
	Green Mountain nat. pol.	do	1	30	15	2	.....	.....	48	Approximately 90 per cent
	Green Mountain check	do	.....	27	15	6	.....	.....	48	

[a] These selections were planted in single hills alternating with Green Mountain; all other seedlings were planted in rows in which 5 hills of the seedlings alternated with 5 hills of Green Mountain.

cross Richter's Jubel × Seedling No. 44537, the probabilities are less than 1 in 100 that they are samples from the same population. There are evidently more factors or more potent factors for resistance involved in the Richter's Jubel × Seedling No. 44537 cross than in selfed Katahdin.

As was to be expected, there was a great difference between severity of scab epidemics in different years. This is shown by the 1936 and 1937 data for 42 white skin seedlings of the cross Richter's Jubel × Seedling No. 44537 and the corresponding Green Mountain checks (Table 5). If the Green Mountain checks for the year 1936 are compared with those for 1937 by the chi-square test, the probabilities are much less than 1 in 100 that they are alike. The various seedling varieties, however, showed about the same degrees of resistance in relation to Green Mountain in 1937 that they did in 1936, the probabilities in each year being less than 1 in 100 that the seedling varieties and the Green Mountain checks were alike in their reactions to scab.

None of the resistant foreign introductions or seedlings developed in the early years of these investigations have approached the commonly grown commercial varieties in vigor and yield, but some of the recent productions resulting from hybridization and selection are more promising. One of these is shown in figure 3. This is a white-skin variety, a selection

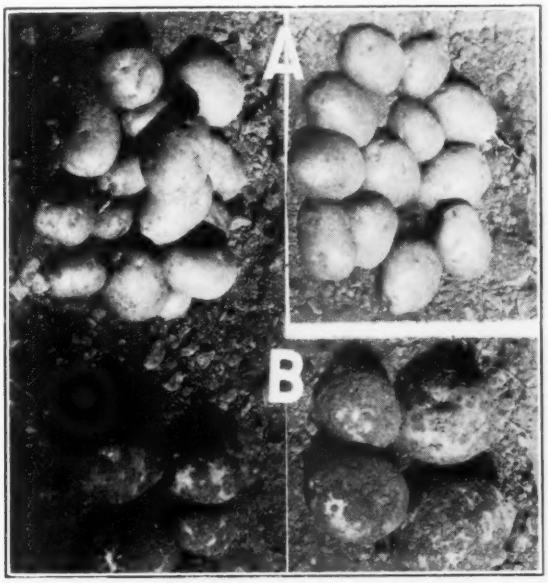

FIG. 3. Two hills of a scab-resistant segregate (A) from the cross, Hindenburg × Katahdin, shown in comparison with two hills of the susceptible Green Mountain check (B), grown approximately 14 inches distant from the seedling hills.

from the cross, Hindenburg × Katahdin. Two hills of the seedling, A in the picture, are shown in contrast to two hills of the Green Mountain variety B grown in alternate hills approximately 14 inches apart in the row. This

TABLE 4.—*Comparison between russet-skin segregates and their white-skin sibs in their reaction to pustule types of common scab*

Pedigree No.	Parents	Skin character	Classes of infection								χ^2 test of russet-skin resistance compared with white
			0	1–20	21–40	41–60	61–80	81–100	Susc. discarded as seedlings	Total	P
				Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*			
× 277	Columbia Russet × S44537	Russet	.....	64	33	6	3	...	61	167	Less than 1 per cent
	do	White	.....	5	2	6	7	4	224	248	
× 295	Russet Rural × S44537	Russet	.....	59	18	.....	.....	...	34	111	do
	do	White	.....	.....	9	20	7	...	201	237	
× 579	Rural New Yorker × S44537	Russet	66	9	2	1	.....	...	27	105	do
	do	White	1	8	13	7	1	...	298	328	
× 528	Richter's Jubel × S44537	Russet	.....	165	40	10	1	...	4	220	do
	do	White	.....	35	90	62	3	...	211	401	
× 574	Arnica × S44537	Russet	.....	10	5	2	.....	...	5	22	Approximately 5 per cent
	do	White	.....	.....	7	3	1	...	37	48	
× 1037	S45208 × S44537	Russet	.....	.....	36	20	1	...	68	125	Less than 1 per cent
	do	White	.....	.....	2	27	16	6	178	229	
× 920	S44537 × S46422	Russet	.....	50	13	3	.....	...	51	117	Less than 1 per cent
	do	White	.....	.....	1	16	2	1	133	153	
Inb. 1364	S44537 selfed	Russet	16	10	1	.....	.....	...	.....	27	Exceeds 30 per cent
	do	White	8	23	7	1	.....	...	.....	39	

seedling is all that can be desired from the standpoint of resistance under the conditions of this test, but it sets too many tubers for ordinary growing conditions and the tuber shape is not the most desirable.

TABLE 5.—*Resistant white-skin seedlings selected from the 1936 test and tested again in 1937*

Pedigree No.	Parents	Year	Classes of infection						Total no. seedlings
			0	1–20	21–40	41–60	61–80	81–100	
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
×528	Richter's Jubel ×344537	1936	.....	.....	30	12	.....	.....	.....
	Green Mountain check	1936	.....	.....	.....	.....	15	27	42
×528	Richter's Jubel ×S44537	1937	18	21	3	.....	.....	.....	42
	Green Mountain check	1937	.....	2	5	25	10	.....	42

DISCUSSION AND SUMMARY

Although more research will be necessary before a genetic interpretation of the inheritance of common scab resistance in potato can be made, a number of facts of importance to the potato breeder have been demonstrated in the present investigation. Green Mountain apparently breeds true for susceptibility to scab. Katahdin is susceptible but carries at least one factor for scab resistance in a heterozygous condition. Hindenburg and Ostragis are probably homozygous for resistance. Seedling No. 44537 and Richter's Jubel are heterozygous for scab resistance. There is a genetic linkage between russeting and resistance. In one cross the resistant factors were independent of red and white tuber colors. Russet, red, and white-skin types have been produced that are highly resistant to scab.

A number of white-skin seedling potato varieties have been produced by hybridization and selection that are highly resistant to scab under conditions of the tests at Presque Isle, Maine. Some of these approach commercial varieties in vigor and yield (Fig. 3), but are inferior in other characters. There is every reason to believe, however, that by using these resistant sorts as parents, and by selfing, sib-mating, and back-crossing, new varieties superior to the commonly grown commercial sorts can be produced.

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FURTHER STUDIES ON HOST RELATIONSHIPS OF PEACH MOSAIC IN SOUTHERN CALIFORNIA[1,2]

L. C. COCHRAN AND LEE M. HUTCHINS

(Accepted for publication Aug. 23, 1938)

Mosaics of almond, apricot, plum, and prune, occurring naturally in southern California, have been reported by the writers to produce peach-mosaic-like symptoms in peach after graft inoculations.[3] In the paper here presented, further observations and experiments on host relationships of peach mosaic are reported, emphasis being given to results of inoculations of peach mosaic into almond, apricot, plum, and prune.

Healthy apricot nursery trees were inoculated by budding from typically mosaic-affected commercial J. H. Hale peach trees, in September, 1937. In all cases, the inserted peach buds (inoculum) made union, and growth from these buds showed severe peach-mosaic symptoms in the spring of 1938, but no mosaic symptoms had been manifested in apricot growth of these trees up to the time this paper was prepared (June, 1938). In the spring of 1938, healthy J. H. Hale test-scions were grafted on limbs of these trees a few feet from the point of inoculation. The test-scions developed symptoms of peach mosaic shortly after growth started, showing that under the conditions of the experiment peach-mosaic virus was able to pass through the apricot trees, after artificial inoculation, without producing symptoms in this species. In healthy plum and prune nursery trees that were inoculated as were the apricot trees, growth from the mosaic-infected peach buds (inoculum) showed typical symptoms of peach mosaic throughout the spring of 1938, whereas the plum or prune growth of these trees showed no mosaic symptoms. Healthy J. H. Hale test-scions grafted on these trees in the spring of 1938, a few feet from the point of inoculation, behaved variously. Up to June 9, about 75 per cent of these scions had developed typical peach mosaic and about 25 per cent were still normal. It is possible that the virus passed more slowly through plum and prune than it did through apricot, and that all of these peach test-scions on plum or prune eventually may show peach mosaic symp-

[1] Paper No. 390, University of California Citrus Experiment Station and Graduate School of Tropical Agriculture, Riverside, California.

[2] Cooperative investigations between the University of California Citrus Experiment Station and the Bureau of Plant Industry of the U. S. Department of Agriculture.

[3] Cochran, L. C., and Lee M. Hutchins. Peach-mosaic host-relationship studies in southern California. (Abstract.) Phytopath 27: 954. 1937.

toms. In check experiments, where healthy almond, apricot, plum, and prune nursery trees were not inoculated with mosaic-infected peach buds but were indexed with healthy J. H. Hale test-scions, growth from all test-scions was normal throughout the spring of 1938, showing that no latent virus capable of producing peach-mosaic-like symptoms in peach was present in the trees before the experiments were set up.

Nonpareil almond nursery trees, on which peach-mosaic-affected J. H. Hale scions from diseased orchard trees were grafted by H. S. Reed, in 1935, have developed no symptoms of peach mosaic in the almond growth, even in cases where growth from the peach scion (inoculum) has attained 4 to 5 feet in length and has shown severe peach mosaic symptoms during the period 1935–1938. Healthy J. H. Hale peach test-scions, grafted on the almond wood of these trees in 1937 or 1938, have developed characteristic symptoms of peach mosaic, showing that the virus may be present in the almond for at least 3 years without producing mosaic symptoms.

When buds or scions from orchard apricot trees showing typical apricot mosaic as it occurs naturally were grafted on J. H. Hale peach nursery trees, symptoms identical with peach mosaic in J. H. Hale were produced. Inoculation back to apricot nursery trees, through grafts from the J. H. Hale trees thus infested, produced typical apricot mosaic in the apricot trees. Similar experiments with naturally occurring almond, plum, and prune mosaics are in progress but are not sufficiently mature for records.

Naturally occurring mosaics of almond, apricot, and prune were transmitted from diseased to normal trees of the respective species, the incubation period being similar to that for peach mosaic in peach. Naturally occurring almond mosaic in orchard trees, when inoculated into healthy apricot nursery trees, produced in the latter species symptoms typical of apricot mosaic.

Surveys of commercial plum and prune orchards of large trees, contiguous to commercial peach orchards in which 75 per cent of the peach trees showed peach mosaic, revealed no mosaic symptoms in the plum or prune trees. In 5 such instances, rows of plum and prune trees were indexed by grafting on each tree a healthy J. H. Hale peach scion, or by grafting a bud from each orchard tree into a healthy J. H. Hale peach nursery tree. The test-scions and the nursery trees grew normally in all cases and showed no mosaic symptoms, indicating that peach mosaic had not spread naturally into the plum or prune trees in these instances. Indexing of the plum part of trees resulting from topworking mosaic-affected peach trees with plum scions showed the peach-mosaic virus present in the plum top in all cases. Surveys of old French Prune orchards contiguous to apricot and almond orchards heavily infected with naturally occurring mosaics of these species revealed that only 1 to 2 per cent of the prune trees were mosaic-affected. Indexing of these orchards by budding from each tree to a healthy J. H. Hale nursery tree showed that infection was present in the prune trees only where the latter showed symptoms.

These data, shown graphically in figure 1, establish the fact that, while

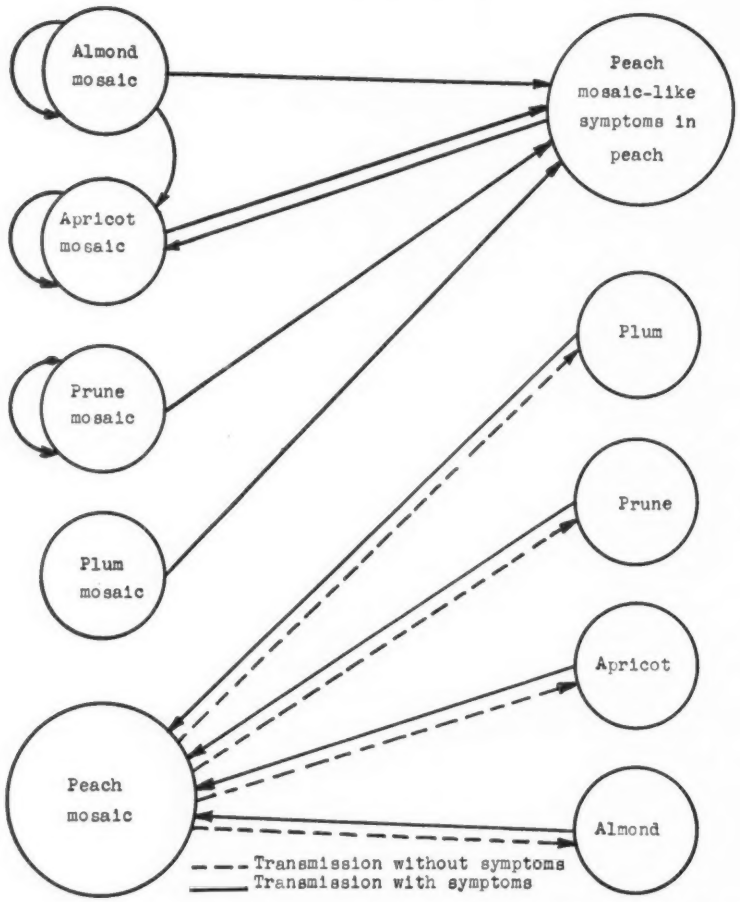

FIG. 1. Graphic outline showing results of cross-inoculation studies with naturally occurring stone fruit mosaics in southern California, as far as completed by June, 1938.

certain naturally occurring mosaics of almond, apricot, and plum are capable of producing peach-mosaic-like symptoms in peach by means of graft inoculations, a reciprocal relationship may not be true. When naturally occurring peach mosaic in peach is inoculated into almond, apricot, and plum, these species may act as symptomless carriers of the peach-mosaic virus.

While this evidence would seem to indicate that more than one mosaic-producing virus of *Prunus* species may be present in southern California, it is difficult to explain why all of these mosaics should produce symptoms in peach indistinguishable from peach mosaic in peach. The difficulty is further increased by survey reports indicating that the natural distribution of these mosaics in southern California is correlated in a general way with the presence of mosaic-affected peach trees. The possibility of virus strains must be considered, and the authors feel that further experimentation will be necessary before definite conclusions can be drawn.

A NEW ENTOMOGENOUS FUNGUS ON THE CORN EARWORM, HELIOTHIS OBSOLETA

VERA K. CHARLES

(Accepted for publication July 22, 1938)

While cooperating in the study of the fungus, *Sorosporella uvella* (Krass.) Gd., parasitic on the corn earworm, Mr. F. F. Dicke, Bureau of Entomology and Plant Quarantine, uncovered fungus-infected pupae from hibernation cages at the Arlington Experimental Farm of the Bureau of Plant Industry at Arlington, Virginia. These specimens appeared to be very different from those attacked by *Sorosporella uvella*, and a microscopic examination showed this to be true.

The first collection was made in May, 1936, and subsequent collections from the same locality in May and November of 1937, and in March, 1938.

Specimens of this fungus were also collected by Mr. Dicke at Moorestown, New Jersey,[1] and McLean, Virginia, in March, 1938. Material was also received from Mr. A. F. Satterthwait of the Bureau of Entomology and Plant Quarantine, in 1937. This collection was made at 2 canneries at Hoopeston, Illinois, where the worms had been observed to be dying.

MACROSCOPIC APPEARANCE OF THE DISEASED INSECTS

There was no external evidence of the fungus in most of the spring collections of diseased pupae. The color was normal and the bodies remained unshrunken. In a few instances, however, the fungus could be seen emerging from the sutures and forming a cobwebby mass on the exterior of the body. Specimens of diseased pupae collected in the fall were frequently found encased in a smooth, white, mycelial sheath. The presence of a similar development has been observed in other species of entomogenous fungi (Fig. 1). Fron,[2] in describing *Spicaria verticillioides*, which

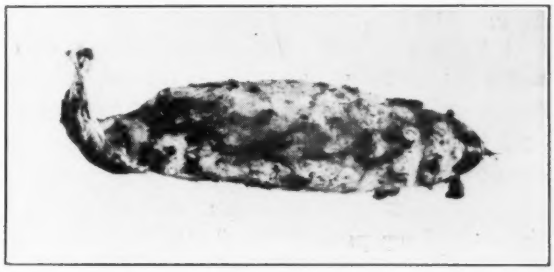

FIG. 1. Pupa of corn earworm covered with a mycelial sheath of the invading fungus. ×2.

[1] The larvae from which the pupae developed at Moorestown were collected in Fairfax County, Virginia, and placed in hibernation cages at Moorestown, New Jersey, in September, 1937. There is a possibility that the fungus was present in the larval stage, and transported to Moorestown.

[2] Fron, G. Note sur quelques Mucédinées observées sur *Cochylis ambiguella*. Bull. Soc. Myc. France **27**: 482–487. 1911.

he later transferred to *Spicaria farinosa verticillioides*,[3] stated that the chrysalis presented the appearance of being covered with a white mold.

GENERAL DISCUSSION

No fungus fructification was observed on any freshly collected diseased pupae. The bodies of the pupae, however, contained a mass of tightly packed mycelium that had caused the complete obliteration of nearly all the organs. The alimentary canal alone was uninvaded.

In the younger material there was observed, in addition to the well-developed mycelium, blastocysts, similar in morphology to those described by Speare[4] in his studies of *Sorosporella uvella*.

Neither the mycelium filling the bodies of the pupae nor that from the sheath surrounding them gave a clue to the identity of the fungus parasite. It was, therefore, necessary to culture the material in order to ascertain whether the mycelium in the bodies of the pupae and that producing the mycelial sheath represented identical or different fungi and to determine the taxonomic position of the fungus or fungi involved.

CULTURAL CHARACTERS

Cultures of the fungus were made from the mycelium filling the body cavity and from the mycelial sheath. The organism grew readily on various media, but the type of growth differed consistently according to the kind of media used. On Molisch, potato, and corn-meal agar, in both Petri dishes and test tubes, the growth was white, sometimes zonate and slender clavae were produced (Fig. 2, A and B). The latter when quite mature adhered to the sides of the test tube or Petri dish, and showed a slight yellowish tinge. The clavae produced in the center of the cultures remained white and showed no coloration whatever. In cultures grown on wort, the clavae were broadly fan-shape and flattened (Fig. 2, C).

The method of fructification was distinctive, according to the culture medium on which the fungus was grown.

On corn meal, potato agar, and Molisch the fructification was developed uniformly over the entire surface of the clavae, while, on wort, the fungus fruited principally at the base of the clavae and only sparingly along the length of the clavae. Fructifications developed from the mycelial sheath also were produced in this manner.

A very luxuriant development of mycelium was produced from pupae encased in the mycelial sheath and kept at a low temperature in a tin container lined with moist filter paper. At first this growth was snow-white and byssoid, but gradually produced irregular flabelliform clavae. As in the wort cultures, the conidiophores were developed more frequently on the smaller clavae, and sparingly over the surface of the growth (Fig. 3, A

[3] Fron, G. Sur une Mucédinée de la Cochylis. Bull. Soc. Myc. France **28**: 151–154. 1912.

[4] Speare, A. T. Further studies of *Sorosporella uvella*, a fungus parasite of noctuid larvae. Jour. Agr. Res. [U. S.] **18**: 399–440. 1920.

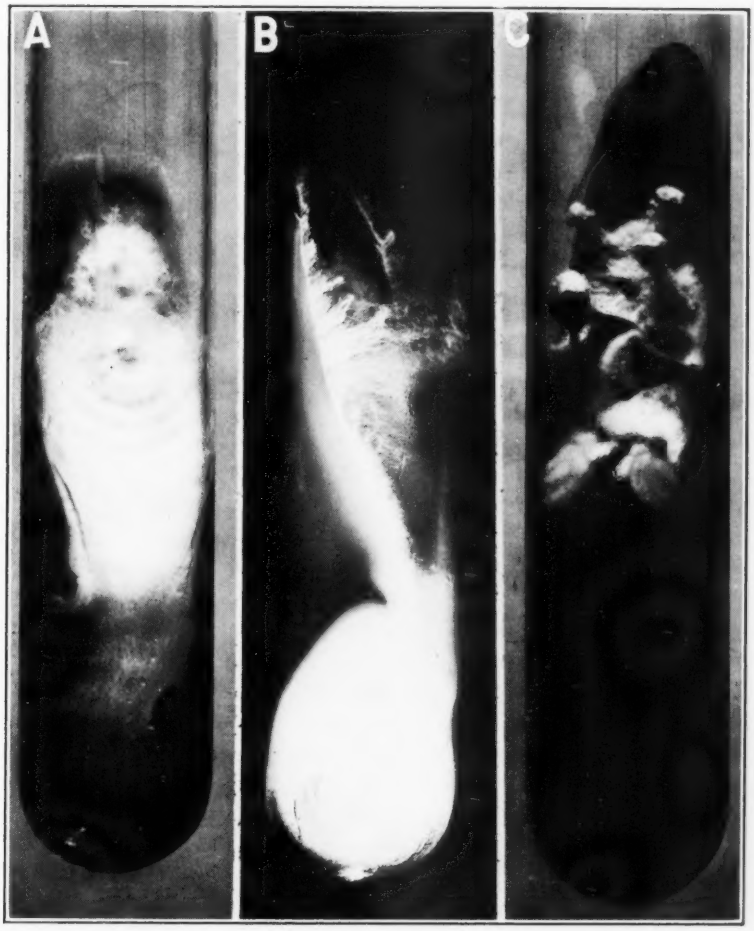

FIG. 2. Cultures of the corn earworm fungus. $\times 1\frac{1}{2}$. A. On Molisch showing zonate growth. B. On Molisch showing development of slender clavae. C. On wort showing fan-shape clavae.

and B). The fungus on the pupae in the tin boxes grew luxuriantly and continuously for over 5 months, with only the addition of a few drops of water from time to time to keep the filter paper slightly damp. The fungus, developed from the pupae in the boxes, remained snow-white for over 6 weeks, when it began to show a slight lemon color.

IDENTITY OF THE FUNGUS

Very few species of fungi have been reported as occurring on the corn earworm, either as parasites or as secondary agents. A search of the literature failed to locate a species identical with the fungus occurring on the pupae of *Heliothis obsoleta*. The type of fructification is typical of the genus *Spicaria*, but, as in the case of certain other entomogenous fungi, normally classed as Mucedinaceae, the formation of synnemata places the fungus among the Stilbaceae. This fungus has been cultured continuously

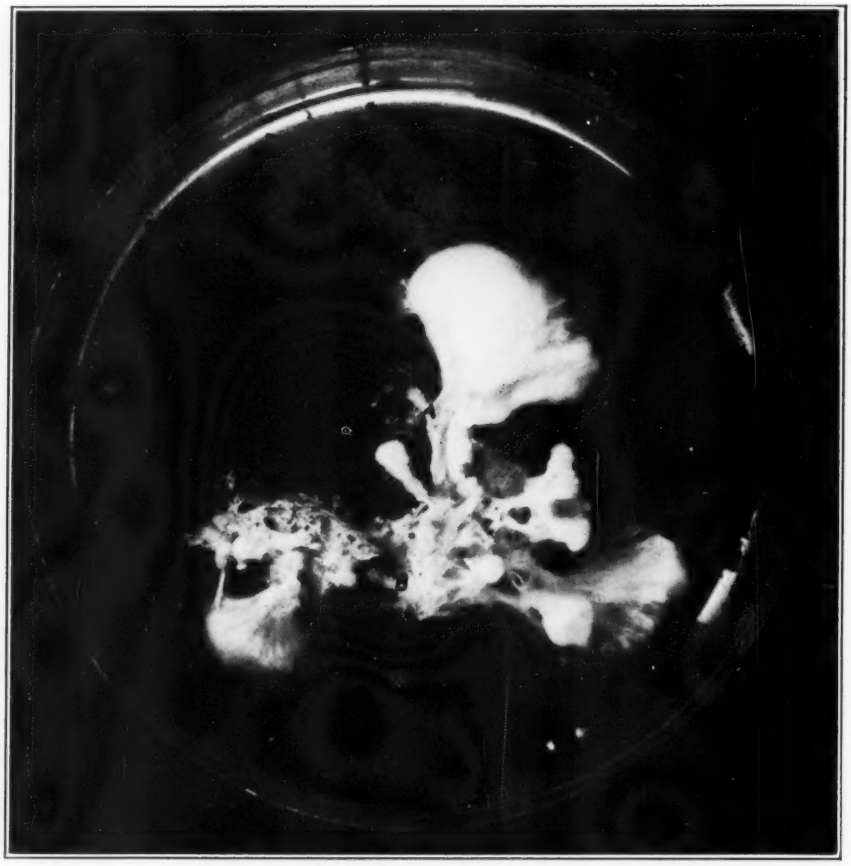

FIG. 3. Culture developed from the mycelial sheath of the invading fungus; conidial formation on small clavae A and B. Photographed by M. L. F. Foubert.

for over 2 years in the hope of developing an ascogenous stage, but only the conidial form has been produced. In 1934 a specimen of *H. obsoleta* was received from Richmond, Virginia, which bore a prolonged sterile club suggestive of the genus *Cordyceps;* but there was no evidence of perithecia or remains of a conidial stage with which to make comparison.

Several species of *Spicaria,* some under the generic name of *Isaria* have been described on insects. Of these species *Spicaria farinosa verticillioides* approaches the form on *Heliothis* most closely, but it is believed that the fungus considered here is sufficiently distinct to be considered a new species.

DESCRIPTION OF THE FUNGUS

The color of the mycelium present in the bodies of the pupae was white to cream and consisted of segments of one to several cells. The latter presented considerable variation in length, but were fairly uniform in diameter. The blastocysts were observed intermingled with the mycelium, intact or in the process of germination.

As previously mentioned the development of synnemata seemed to be influenced by the kind of media on which the fungus was grown. On wort the clavae were flabelliform, broader, and more effuse than when grown on Molisch, potato, or corn-meal agar, and measured from 1 to 1½ cm. in height and were fully as broad in diameter. In addition the fructification was sparingly developed and more generally basal than over the entire surface of the synnemata. In the cultures grown on Molisch, potato, and corn-meal agar, in both test tubes and Petri dishes, the fructification was more typically stilbaceous, and the conidiophores developed uniformly over the entire surface of the synnemata. On these media, single or branched synnemata were developed on the sides of the tubes or Petri dishes and ranged from 1 to 2 cm. in height. The surface growth was pure white and more or less zonate (Fig. 2, A).

In the study here reported, the fungus presented considerable variation. An effort has been made to cover these differences in the specific description.[5]

On potato and corn-meal media the method of fructification was very typical of the genus *Spicaria*. The conidiophores bore 3 to 8 whorls of phialides and long chains of spores. As the cultures matured the number of whorls became fewer and more irregular and the verticillate arrangement less marked. The generic name of the host *Heliothis* has been used for the specific name of the fungus, which is accordingly designated **Spicaria heliothis,** n. sp.

Spicaria heliothis

Mycelium white, septate; synnemata solitary or gregarious, 1–1.5 cm. high; conidiophores simple or branched, erect, bearing 3–8 whorls of prophialides 3–5 verticillate or irregularly grouped, oblong, 7–8 μ; phialides globose with short sterigma; conidia variable in shape, ovate-elliptical, in long chains, 3–3.5 × 5–7 μ.

Mycelia albo, septato; synnematibus solitariis vel aggregatis, 1–1.5 cm altis; conidiophoris simplicibus vel ramosis erectis, 3–8 verticilla prophialidium gerentibus; prophialidibus 3–5 verticillatis vel conglomeratis, oblongis 7–8 μ; phialidibus globosis sterigmatibus breviusculis; conidiis variabilibus, ovato-ellipsoideis, in catenulas longas, 3–3.5 × 5–7 μ.

In culturis ex Heliothe obsoleta.

Type deposited in the Mycological Collections of the Bureau of Plant Industry.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

[5] It may avoid confusion in any future study of the genus *Spicaria* on *Heliothis* or other insect hosts, to call attention to the fact that during the course of the present investigation several species of this genus were encountered on the corn earworm. One very striking form, observed only once, was a robust, loosely branched type, having large phialides and spores. Another species, encountered only once, was a small and delicate form, pale vinaceous fawn in color. In addition to these 2 forms, an isolation was made from one of the Hoopeston, Illinois, collections, typical of *Spicaria farinosa verticillioides*. This species was not isolated from insects with the fungus discussed in this paper, but appeared to be the only parasite of the material from which it was cultured.

THE 1938 CROWN RUST EPIDEMIC OF OATS IN ARKANSAS IN RELATION TO HYBRIDS OF BOND AND VICTORIA

H. R. Rosen and L. M. Weetman

(Accepted for publication July 16, 1938)

In the December 1937 meeting of The American Phytopathological Society, Moore, Downie, and Murphy[1] reported a new race of crown rust, *Puccinia coronata avenae* F. and L., that attacks Bond oats (to be designated race 45, according to communications between H. C. Murphy and the senior writer). This race appeared on Bond and its hybrids at the Minnesota Agricultural Experiment Station and, according to the authors, was brought in, either on 2 collections made in Texas in the spring of 1937, or on material representing race 1, sent by H. C. Murphy to the Minnesota Station.

What appears to be the same race was found and identified by the present writers in Arkansas in 1937. In the process of identifying the races of crown rust present in Arkansas, from collections gathered by the writers in May and June, 1937, in all of the important oat-growing counties of the State, 2 single-pustule inoculations were made in October, 1937, on Markton oats from Collection No. 129, gathered on Bond at the Arkansas Experiment Station farm, Fayetteville. On November 17, when the second series of single-pustule inoculations on Markton were fully developed, the duplicate cultures were inoculated on the series of differential varieties that Murphy[2] had selected for the identification of races of crown rust. Both of these cultures produced within 10 to 14 days, a type-4 reaction on all the differentials, including Bond, except Glabrota, which yielded an I reaction, and Victoria, which showed a slightly greater susceptibility to this collection than it does to most collections of race 1. To make certain of these reactions, the duplicate single-pustule cultures were inoculated again on all the differentials and the same results obtained. It was apparent that a race of crown rust had been found that attacks Bond severely, and is mainly distinguished from race 1 by its reaction on this variety.

Out of 153 collections, race 45 subsequently was isolated in duplicate from 25 of them, while in 41 additional collections it appeared either in one or both of the duplicate isolations in amounts on Bond suggesting greenhouse contamination. The counties in which race 45 appeared in duplicate from single collections and apparently free from contaminations, were Carroll, Clay, Crawford, Cross, Franklin, Lee, Madison, Marion, Poinsett, and Washington. Most of these are in northern Arkansas.

This new race, and race 1, was inoculated on some 3,700 Victoria hybrid

[1] Moore, M. B., A. R. Downie and H. C. Murphy. A new race of crown rust that attacks Bond oats. (Abstract.) Phytopath. 28: 16–17. 1938.

[2] Murphy, H. C. Physiologic specialization in *Puccinia coronata avenae*. U. S. Dept. Agr. Tech. Bull. 433. 1935.

plants grown in the greenhouse, while a similar number of Bond hybrids were inoculated with race 1 only. Within several weeks contaminations with the new race became increasingly common on all the Bond hybrids, despite serious efforts to prevent them. Lack of greenhouse space made it impossible to segregate the Bond hybrids from the others and to keep the inoculated plants and incubating chambers in rooms separated from healthy plants. There appeared to be good evidence for the conclusion that under greenhouse conditions race 45 is far more aggressive than race 1.

Contamination of these Bond hybrids with race 45 was most discouraging from the standpoint of breeding for crown-rust resistance. Although overhead watering was carefully avoided and the leaves kept free from water, many of the plants suffered a loss of 50 per cent or more of their leaf areas through rust infections before and during heading. The ease with which this race spread in the greenhouse seemed ominous for Bond and its hybrids, under field conditions.

Fortunately for the breeding program, the 1938 season offered almost ideal field conditions at Fayetteville, Arkansas, for natural dissemination and infection, so that Bond and its hybrids, as well as all other varieties, were subjected to a severe epidemic of crown rust. There were present several thousand F_2 Bond and Victoria hybrids that had overwintered successfully, as well as over 150,000 F_2 and F_3 plants of the same hybrids from seed sown in March.

Crown rust was first found in the experimental plots on April 25, on Lee, one of the common and best types of winter oats for this region. Infections were very few; in fact, they were not abundant until the second week of May. With the continuation of excessive rains extending through April, May, and early June, crown rust assumed epidemic proportions so that by May 24 such varieties as Lee, Custis, Winter Turf, Hairy Culberson, and Oklahoma selections of Hairy Culberson × Fulghum (Winter type), showed nearly 100 per cent infection of the areas of leaves 10 or more days old. From that date until harvest—about 3 to 4 weeks later in the winter oats—as soon as the leaves became old enough to show infections, rust occupied almost all of the blades and much of the sheaths.

All Victoria hybrids, resistant and susceptible plants alike, suffered severely from crown rust. The writers' resistant Victoria hybrids, as well as some of the promising ones of Stanton and Murphy (U. S. Department of Agriculture) were so completely spotted and rusted that, compared with varieties of the Red Rustproof group, the superiority of the Victoria rust-resistant plants remains to be shown, so far as yield, weight, or other desirable character is concerned.

As a whole, even the most resistant plants—judged by the presence of minute pustules or the complete absence of pustules on the lowermost leaves and the presence of necrotic and chlorotic spots—showed numerous pustules on the upper leaves during heading, many of the pustules being moderately large and yielding a 2 to 3 type reaction. In this region Vic-

toria hybrids that show a high degree of resistance in the seedling stage become more susceptible as the plants advance in growth and maturity.

In contrast to the appearance of all Victoria hybrids under field conditions and to the Bond hybrids grown in the greenhouse, the Bond hybrids

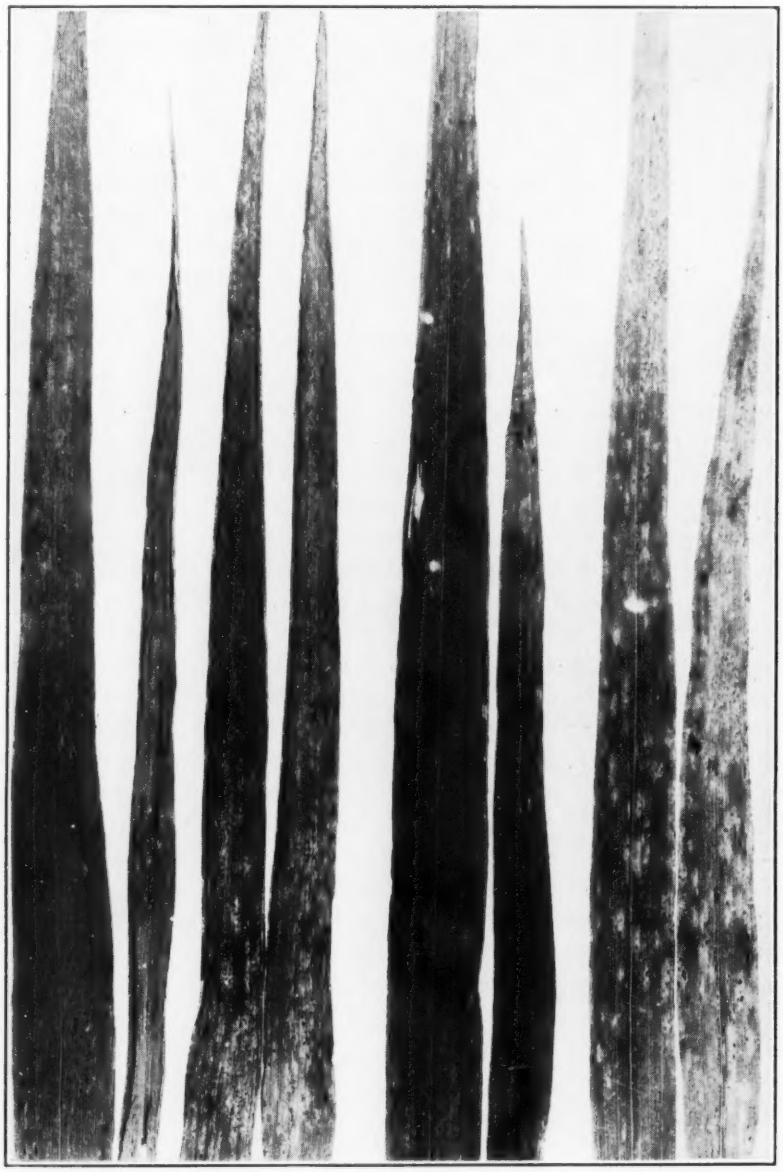

FIG. 1. Comparison between 2 leaves each of Bond, Victoria, resistant Bond hybrid, and resistant Victoria hybrid (left to right), in amount of rusting and spotting. Leaves gathered June 16, 1938, at Fayetteville, Arkansas. Note the much greater number of pustules and chlorotic-necrotic spots on Victoria and its hybrid than on Bond and its hybrid, typical of reactions in 1938.

in the field in 1938 offer much encouragement, so far as crown rust resistance is concerned (Fig. 1). Even up to the late dough stage, none of the Bond hybrids, resistant to race 1 in the winter-oat plots, showed as much as 5 per cent of the leaf area infected with race 45. In the spring-oat plots there were a few instances in which this race was present in such abundance as to interfere with the identification of plants resistant to race 1. In by far the largest number of cases there was no difficulty in detecting resistance to race 1 in these spring oats, since race 45 was present in a very limited number of infections, in contrast with plants susceptible to race 1 which showed fully 90 to 100 per cent of the leaf area infected at heading. In addition, the abundant spotting of leaf areas in Victoria hybrids reacting to race 1, was absent in Bond hybrids.

There are 2 possible explanations for the difference in the response between greenhouse and field-grown Bond hybrids when subjected to race 45 of crown rust. 1. This race makes its appearance in the field in northern Arkansas considerably later than race 1. 2. Bond hybrids escape race 45 in much the same manner that Red Rustproof oats escape this race and race 1 in the field. In the greenhouse the latter show abundant infections and a completely susceptible reaction to both races in the seedling stage, as well as in the later stages, a fact previously noted by several investigators.

It is conceivable that if Bond hybrids were grown extensively in the South, race 45 might become as destructive as race 1 now is. However, the fact that race 45 was found in at least several collections in this region in 1937 and as late as November on potted oats placed outdoors during the summer, and the further fact that both fall- and spring-sown Bond hybrids showed a much reduced amount of infection with race 45 in 1938, compared with plants susceptible to race 1, suggest that race 45 on such hybrids is not much more menacing in Arkansas than race 10 of *Puccinia graminis avenae* is to the Richland oat and its hybrids in the Middle West. However this may be, in the presence of the severe crown-rust epidemic of 1938, Bond hybrids appeared to be far more promising than those of Victoria in their reaction to the races of rust prevalent in northern Arkansas. This duplicates reactions noted on the parent varieties Bond and Victoria in 1935, the other recent year in which crown rust was present in this region in epidemic proportions.

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THE SEPARATION OF PLANT VIRUSES BY CHEMICAL MEANS[1]

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INTRODUCTION

The differentiation of plant viruses has received considerable attention in recent years and a number of distinct viruses and strains have been described. The criteria used have been based largely upon symptomatology, differential hosts, selective insect transmission, and physical properties. Obviously it is not only desirable to distinguish these viruses but to separate or isolate them at will from other viruses with which they may sometimes be associated. Such mixtures are fairly common in nature and in accidental contaminations in experimental work. Separation is, in some instances, relatively simple when based upon the known criteria; however, in other cases it may be extremely difficult or impossible. For example, it may be possible to isolate one component of a mixture by thermal inactivation or by means of a susceptible host but especially difficult or time consuming to isolate the other component.

The differential action of chemicals upon viruses may offer many possibilities in virus separation due to the great variety of kinds and concentrations of chemicals available. A survey of this field has been made with the hope that chemicals might be found that would prove especially useful for the purpose in mind. The results have been promising to the extent that it has definitely been established that chemicals have specific action on viruses. In several instances both components of virus complexes have been isolated by differential tolerance to chemical substances. The data and conclusions of this investigation are presented in this paper.

REVIEW OF LITERATURE

The inactivating action of toxic chemicals upon plant viruses has been investigated intermittently since the work of Koning (12) and Beijerinck (3) in 1899. The purpose of these early investigations was largely to secure some clue as to the nature of the viruses. Allard (1) was the first to make a special study of the inactivation of viruses by chemicals. He treated the virus of tobacco mosaic with a wide variety of germicides, salts, acids, etc., and concluded that this virus was remarkably resistant. These earlier experiments were all with the highly tolerant virus of ordinary tobacco mosaic. More recently other plant viruses have been shown to be much less resistant to chemicals than the tobacco-mosaic virus. Unfortunately, the

[1] This investigation was conducted under allotments from the University of Wisconsin Research Fund. The paper resulting therefrom, and here presented, formed part of a thesis presented to the Graduate School of The University of Wisconsin in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

The writer is indebted to Dr. James Johnson for advice and criticism during the work and preparation of the manuscript.

results of the different investigators are not readily comparable because a standard technique has not been used. It is impossible, therefore, from the available data, to draw satisfactory conclusions concerning the actual tolerance of the different viruses to the chemicals tested.

Little has been done on the actual separation of mixtures of viruses by chemicals. Johnson (8) earlier expressed the belief that inactivation by chemicals would be useful in separation and classification of viruses. He treated many potato and tobacco viruses with alcohol and nitric acid (9) and thereby showed that differentiation, at least to some extent, was possible by this method. A few cases have been reported where one component of a virus mixture has been isolated by chemical inactivation. Shapovalov (13) treated the virus combination causing tomato streak (*i.e.*, ordinary tobacco-mosaic virus and latent potato virus) with a wide variety of chemicals. He was able with some constancy to isolate the ordinary tobacco-mosaic virus, but he found the isolation of the latent potato virus much more difficult and entirely unpredictable with the chemicals tested. He divided the chemicals into 3 groups according to their effect upon the viruses as follows: (a) those like the bile salts and boric acid, which had little or no effect upon either virus; (b) those having a strong inactivating effect upon both viruses, such as copper sulphate and sulphurous acid; and (c) those that were good inactivators but had no special affinity for either virus, *e.g.*, nickel sulphate and cobalt sulphate. This latter group was found to behave erratically, with the result that either virus might be inactivated first. Folsom and Bonde (5) treated the potato rugose-mosaic virus complex with several chemicals, but did not observe differential action or secure separation of the viruses. Freeman (6) has reported the separation of the latent mosaic virus from the potato rugose-mosaic virus complex by changing the hydrogen-ion concentration.

Stanley and Wyckoff (15) found that the tobacco-mosaic virus was more tolerant to high hydrogen-ion concentrations than the tobacco ring-spot virus, but that the latter may be more tolerant to low hydrogen-ion concentrations. It is not known whether separation by this method has been accomplished. Gratia and Manil (7) and Chester (4) have suggested that neutralization reactions of specific immune sera may be useful in the separation of virus mixtures. Stanley and Wyckoff (15) claim to have isolated the tobacco ring-spot virus from a mixture with the tobacco-mosaic virus by neutralizing the latter with specific immune serum. The author has recently published a preliminary note (2) in which the viruses of cucumber mosaic and potato ring-spot were each reported to be isolated by chemical means from a mixture. Further details of this and other separations are given in this paper.

MATERIALS AND METHODS

The viruses used in the present studies were those of ordinary tobacco mosaic (*tobacco virus 1*), ordinary cucumber mosaic, tobacco ring spot, potato ring spot, potato veinbanding, and tobacco streak. These viruses

were selected because of their availability and the wide range of physical properties they represent.

Infective extracts for treatment were obtained by extraction from diseased tobacco plants (*Nicotiana tabacum* L. var. Havana-Seed). Five to 10 plants showing typical disease symptoms were macerated with mortar and pestle and extracted through cheesecloth. The extracts were again filtered through cheesecloth to remove coarse particles of plant débris. Since the primary purpose of this investigation was to separate the viruses, and only a few of the viruses concerned have been purified to any extent, no attempt was made at their purification. The chemicals were dissolved in distilled water at twice the concentrations to be tested and then mixed with equal quantities of infective plant extract. Usually 2 cc. of the extract were mixed with 2 cc. of the chemical solution in a test tube. This gave the desired concentration of the chemical and, at the same time, diluted the virus by one half. The preparations were then held in a water bath at 20–22° C. for 1 hour, after which 1 cc. samples from each were pipetted into evaporating dishes and 50 cc. portions of distilled water were added. This procedure brought all the samples of virus extract to a dilution of 1–100 and diluted the most toxic substances sufficiently to prevent chemical injury on the inoculated plants.[2] The infectivity of each treated extract was determined by inoculation of 5 small tobacco plants (*N. tabacum* L. var. Havana-Seed) by rubbing the leaves with cheesecloth saturated in the treated preparation.

The chemicals employed represent a wide variety of compounds as follows: salts of heavy metals, heavy metal-organic compounds, phenolic compounds, oxidizing agents, reducing agents, acids, and bases. The effect of 35 chemicals that were tried on viruses are reported in this paper. Many other compounds were tried in a preliminary manner, but their use was discontinued because of inconsistent behavior. A special attempt was made to select fairly stable compounds that were known to have antiseptic properties. Ordinary laboratory grades of chemicals were used, most of them being of analytical grade, but many compounds were commercial preparations as obtained from pharmaceutical houses. The strengths of the chemical solutions are given in percentage on a weight-volume basis.

EXPERIMENTAL RESULTS

Chemical Inactivation and Tolerance of Single Viruses

The relative toxicity of different chemicals may be determined quite readily where the viruses used produce local lesions on certain hosts. With other viruses, however, it becomes a laborious task because of the number of plants required to yield significant figures. Therefore, quantitative determinations of the relative inactivating influences have not been attempted in this investigation. A chemical or a chemical concentration was not considered useful for the present purposes unless it gave complete inactivation at

[2] Preparations containing tobacco-streak virus were diluted 1–10 because of the low tolerance to dilution of this virus.

a concentration not too toxic to the inoculated host tissue following a reasonable dilution for the virus. The value of a chemical or a concentration also was determined by the maximum solubility in water, as well as by its previously known lethal action on microorganisms.

TABLE 1.—*A list of chemicals tested on the six plant viruses used in this investigation, but found insufficiently toxic to cause complete inactivation under the conditions of the experiments*

Chemical	Maximum conc. tried; per cent	Approx. pH of treated extract[a]	Reason for not trying higher concentrations[b]
"Mercury oxycyanide"[c]	1.0	7.5	C
Copper acetate	1.0	4.8	B
Zinc sulphate	10.0	4.6	D
Nickel chloride	10.0	4.5	D
Nickel nitrate	10.0	4.5	D
Protargol[c]	1.0	8.2	D
Collargol[c]	1.0	8.2	D
Flumerin[c]	1.0	8.9	C
Merthiolate[c]	1.0	7.5	B
Xeroform[c]	½ saturation	7.1	C
Bismarsen[c]	1.0	7.7	D
Sulpharsphenamine[c]	1.0	7.3	D
Catechol	5.0	6.4	B
Protocatechuic acid	1.0	4.9	C
Pyrogallic acid	5.0	6.9	D
Bile Salts—Fairchild[c]	10.0	7.3	D
Safranin	1.0	6.0	B
Methyl orange	0.5	7.2	C
Ethyl alcohol	50.0	6.1	E
None (check)		6.4–6.9	

[a] pH measurements were made with the glass-electrode method.

[b] B. Higher concentrations gave burning of leaves. C. Maximum solubility reached. D. Concentration attained that should display toxicity on basis of known antiseptic properties. E. Concentration limited by method of experimentation.

[c] Trade name.

Undoubtedly, many of the chemicals listed in table 1 will inactivate viruses, but they do not completely inactivate any of those studied here under the conditions of the experiments. The antiseptic power of mercury oxycyanide (51.7 per cent to 56.0 per cent $Hg(CN)_2$ + 44.3 per cent to 48.0 per cent HgO) is claimed to be greater than that of mercuric chloride; however, it does not react with albumins so readily as the latter. It is of interest to note here that it has little toxic effect upon viruses as contrasted to mercuric chloride (Tables 1 and 2). Protargol (a brand of strong silver protein) and collargol (colloidal silver and silver oxide) are recognized as weak antiseptics, and here appear to have very little if any inactivating influence upon viruses. Flumerin (disodium-2-hydroxy-mercurifluorescein) is a relatively weak antiseptic and did not have significant effect upon the viruses. Merthiolate (sodium ethyl mercurithiosalicylate) is a potent germicide that does not precipitate proteins and has proved of little value as a virus inactivator in concentrations that do not injure the inoculated plant. Xeroform (a basic bismuth tribromphenate of variable composition with mild antiseptic

properties) and 2 compounds containing trivalent arsenic (sulpharsphenamine and bismarsen) were ineffective as virus inactivators. Two phenolic compounds (protocatechuic acid and catechol) were not sufficiently toxic in the concentrations used to give significant inactivation. Pyrogallol, a reductant, was a weak inactivator; its strength as a reducing agent is, however, somewhat impaired by acid solutions. Since the extracts when treated with pyrogallol gave a test of approximate pH 5.0, this may account for its weak action. The bile salts (Bile Salts—Fairchild, a mixture of equal proportions of sodium glycocholate and sodium taurocholate) are reported to have mild antiseptic action; they appear here, however, to be poor virus inactivators. Safranin was unsatisfactory in this study because it is extremely injurious to plant foliage. Ethyl alcohol has been reported in many cases as a virus inactivator; its action, however, is too slow to be of value in experiments where 1 hour is the standard time for treatment.

TABLE 2.—*A list of chemicals found to be good virus inactivators, and the minimum concentration of each required to completely inactivate the respective viruses*[a]

Chemical	Approx. pH of treated extract[a]	Virus used and conc. of chemical (per cent)					
		Tobacco mosaic	Cucumber mosaic	Tobacco ring-spot	Potato ring-spot	Potato vein-banding	Tobacco streak
Mercuric chloride	5.7	3.0+[b]	0.8	0.1	0.5	0.6	0.1
Silver nitrate	5.5	4.0	2.0+	1.0	0.5	1.0	1.0+
Copper sulphate	3.5	6.0+	1.8	2.0	2.5	1.0	0.2
Lithium carbonate	10.5	1.0+	1.0[c]	1.0	1.0+	1.0	1.0
Stannic chloride	2.2	1.1	0.7	0.4	0.5	0.5	0.5
Mercurochrome[d]	8.2	2.0+	0.5	0.5	2.0+	0.5	0.5
Phenol	6.2	5.0[c]	5.0	5.0[c]	5.0	4.0	2.0
Tannic acid	6.3	5.0+	0.5	0.5	0.5	1.5	0.2
Formalin[d]	6.8	50.0+	1.0	1.0	1.0	0.5+	1.0
Oxalic acid	3.5	2.5+	0.6	1.0	1.0	0.6	0.4
Picric acid	5.9		0.6	0.4	0.6	0.4+	0.4+
Nitric acid	1.5	2.0	0.6	0.4	0.9	0.3	0.5
Sodium hydroxide	11.0	0.3	0.2	0.1	0.3	0.2	0.1
Sodium permanganate	8.7	1.5[c]	0.5	0.1	1.0	0.5	0.2
Potassium permanganate	8.7	1.5	0.4	0.4	0.8	0.2	0.2
Potassium dichromate	6.5	1.0+	0.6	0.4	0.7	1.0+	0.2
None	6.4–6.9						

[a] In 1 hour at 20° C. pH measurements were made with the glass-electrode method.
[b] Plus sign indicates inactivation not complete.
[c] Virus was usually, but not always, completely inactivated at the concentration given.
[d] Trade name.

The chemicals listed in table 2 are all good inactivators for 1 or more viruses at the concentrations given, which are the lowest reliable percentages for complete inactivation. As may be expected, some variation exists as to the concentrations required for complete inactivation of individual viruses. The minimum concentration necessary to give complete inactivation is based upon at least 3 trials. In no trial has the required concentration of a chemical been greater than given in table 2. It is believed that the concentra-

tions shown in this table represent in a general way the relative tolerance of the different viruses to the chemicals listed.

No particular types of chemicals used (Table 2) seem to be outstanding in value for virus inactivation, unless acids and bases are so considered. The concentrations of acids and bases reported in this table are quite high and the ranges in the resulting pH are extremely wide. These chemicals all possess germicidal properties and are all capable of denaturing proteins. The mode of action of the chemicals upon the viruses, however, must necessarily remain in doubt as long as the nature of viruses remains in doubt. The treatment of the viruses in unpurified extracts further obscures the nature of the inactivation. However, it is obvious from a casual examination of table 2 that it should be feasible to destroy some viruses in mixtures by chemical treatment without completely destroying others.

Chemical Inactivation and Tolerance of Virus Mixtures

Previous workers have characterized viruses by their tolerances to chemicals in general, possibly with the assumption that the viruses fall into a definite sequence as to their tolerances to all chemicals. This conception is not without foundation, as will be evidenced by an examination of table 2. Tobacco mosaic is outstanding in its tolerance to chemicals and has been recognized as such for many years. In the present investigation, over half the inactivating chemicals were not toxic enough to completely inactivate this virus and, where complete inactivation did occur, the concentrations required were relatively high. A disappointing feature of this study has been the failure, with one exception to be mentioned later, to find substances to which the ordinary tobacco-mosaic virus would prove to be more intolerant than certain other viruses. The tobacco streak virus may be cited as an example of the other extreme in regard to chemical tolerance. In table 2 the tobacco streak virus is shown to have a comparatively low tolerance to chemicals. The other 4 viruses, namely, tobacco ring-spot virus, cucumber-mosaic virus, potato ring-spot virus, and potato veinbanding virus do not differ strikingly, as a whole, in their chemical tolerance, but show significant differences in response to specific chemicals.

Cucumber-mosaic Virus and Potato Ring-spot Virus Mixture. A comparison of the viruses of cucumber mosaic and potato ring-spot in table 2 will show that the former is more tolerant to mercuric chloride, silver nitrate, and stannic chloride, while the latter is more tolerant to copper sulphate, lithium carbonate, mercurochrome, oxalic acid, nitric acid, sodium permanganate, potassium permanganate, and potassium dichromate. The significance of some of the differential tolerances of these 2 viruses as shown in table 2 is still in some doubt; however, repeated tests of several of these compounds have been made with fairly consistent results.

The cucumber-mosaic virus and the potato ring-spot virus are somewhat similar in their physical properties. The thermal inactivation points are very close, and both have a maximum tolerance to dilution of about 1–10,000

when taken from tobacco. They are, however, separable by insect transmission, aging *in vitro,* and by differential hosts. The symptoms caused by the 2 viruses are quite distinct. The potato ring-spot symptoms on tobacco are characterized at first by small necrotic spots, surrounded by a variable number of necrotic rings. About 7 days after inoculation, systemic symptoms appear in the upper leaves and consist of irregular necrotic lines and concentric rings. Ordinary cucumber-mosaic virus manifests itself on tobacco by the production of chlorosis and some leaf distortion. When the 2 viruses are present simultaneously in tobacco, the resulting symptoms are very striking (Fig. 1). In late stages all the leaf tissue and much of the stem are enveloped in a systemic necrosis. These later symptoms are particularly severe under high air-temperature conditions.

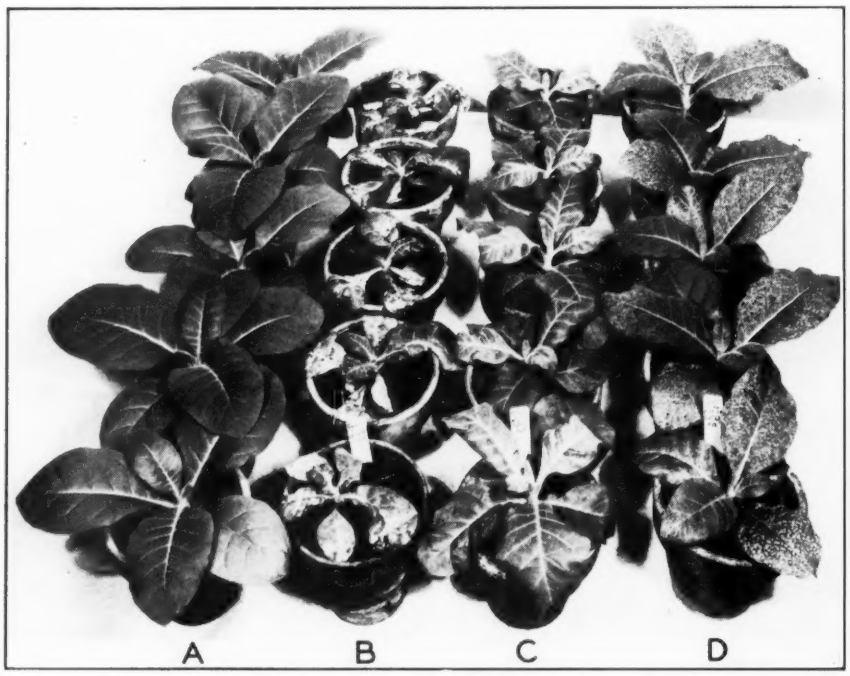

FIG. 1. The separation of the viruses of cucumber mosaic and potato ring spot from a mixture. A. Healthy, noninoculated controls. B. Plants inoculated with the nontreated virus mixture, showing severe stunting and necrosis. C. Plants inoculated with the same mixture treated with 0.3 per cent silver nitrate and showing typical cucumber-mosaic symptoms only. D. Plants inoculated with the same mixture as in B, but treated with 1.0 per cent lithium carbonate and showing typical potato ring-spot symptoms only.

Plant extracts containing cucumber-mosaic and potato ring-spot viruses were prepared as formerly and then combined, giving a mixture of the 2 viruses. This preparation was then treated with a series of concentrations of each of the following chemicals: silver nitrate, mercuric chloride, copper sulphate, lithium carbonate, and potassium permanganate. The conditions

and methods of the experiment were similar to those of the previous section involving inactivation of single viruses.

TABLE 3.—*The separation of the cucumber-mosaic virus and the potato ring-spot virus by chemical treatment from a mixture of the viruses in plant extract*

Chemical	Conc. per cent	Number of infected plants out of five inoculated after treatment of virus mixture			
		with both viruses	with cucumber-mosaic only	with potato ring-spot only	with neither virus
Silver nitrate	0.1	5	0	0	0
	0.2	1	4	0	0
	0.3	0	5	0	0
	0.4	0	5	0	0
	0.5	0	5	0	0
	0.6	0	5	0	0
Mercuric chloride	0.1	5	0	0	0
	0.2	5	0	0	0
	0.3	5	0	0	0
	0.4	5	0	0	0
	0.5	0	5	0	0
	0.6	0	5	0	0
	0.7	0	5	0	0
	0.8	0	0	0	5
Potassium permanganate	0.2	5	0	0	0
	0.3	0	0	5	0
	0.4	0	0	1	4
	0.5	0	0	0	5
	0.6	0	0	0	5
Copper sulphate	1.6	3	0	2	0
	1.7	0	0	0	5
	1.8	1	0	1	3
	1.9	0	0	1	4
	2.0	0	0	2	3
Lithium carbonate	1.0	0	0	5	0
None (check)		5	0	0	0

A typical set of results appears in table 3. Silver nitrate and mercuric chloride completely inactivated the potato ring-spot virus at concentrations that left the cucumber-mosaic virus infectious. Potassium permanganate, copper sulphate, and lithium carbonate behaved in the opposite manner, being more toxic to the cucumber-mosaic virus with the result that the potato ring-spot virus only remained infectious. Figure 1 shows how striking these results appear on the greenhouse bench. Here is shown the isolation of cucumber-mosaic virus from the mixture by treatment with silver nitrate and the isolation of the potato ring-spot virus by treatment with lithium carbonate.

With the exception of the copper sulphate treatment, this experiment has been repeated 10 times. The isolation of potato ring-spot virus with copper sulphate has been repeated only twice, but with similar results in each case.

The conclusion that the viruses in question have been isolated has been proved in many cases by successive inoculations of the isolated viruses to tobacco. In every instance where separation was apparent from the symptoms, the further inoculations confirmed the isolation. In several experiments, the infective extracts for treatment were taken from plants infected simultaneously with both viruses instead of making the combination artificially by mixing extracts from plants infected with the different viruses singly. In other experiments, the extracts were taken from plants that had been inoculated for many days with one virus and following the appearance of symptoms were inoculated with the other only a few days before extraction. In these experiments, the resulting fluctuations in concentrations of the viruses used were not sufficient to alter the results.

Potato Veinbanding Virus and Potato Ring-spot Virus Mixture. A common virus disease of potatoes, known as rugose mosaic, has been shown by Koch (11) and others to be caused by a combination of the latent potato virus and the veinbanding virus. This is a common virus mixture occurring in nature causing considerable injury to potatoes in some districts. The latent potato virus occurs universally in most of the American varieties of potatoes and, as fas as is known, is disseminated in nature solely by the vegetative propagation of the potato. It is, however, readily transmitted by mechanical means. The potato ring-spot virus used in these studies is a strain of this latent potato virus and was isolated by Koch (11). The veinbanding virus is readily transmitted mechanically or by certain aphids, has a slightly lower thermal inactivation point than the potato ring-spot virus, and is less tolerant to aging and dilution. The symptoms on tobacco caused by the veinbanding virus are a slight stunting of the plant, accompanied by a faint vein clearing and vein banding with a drooping of the leaves. The "spot necrosis" symptom on tobacco produced by a mixture of these 2 viruses has been frequently described. Typically, it is a severe stunting with necrotic streaks along the veins and necrotic spots between the veins. It is, therefore, relatively simple to distinguish between tobacco plants infected with the veinbanding virus, the potato ring-spot virus, and a combination of the 2 viruses. There are specific differences between the relative tolerances of these 2 viruses to chemicals (Table 2). The veinbanding virus is more tolerant to mercuric chloride and silver nitrate, while the potato ring-spot virus is more tolerant to most of the other chemicals listed.

The veinbanding and potato ring-spot viruses were artificially combined and treated with chemicals, as was the previously described virus mixture. As shown from the results of a typical experiment given in table 4, the veinbanding virus can be separated from the potato ring-spot virus in a way similar to that of the cucumber-mosaic virus to which it is believed by some to be related. The veinbanding virus again appears to be more tolerant to mercuric chloride than the potato ring-spot virus. Time has not permitted a test on possible separation with silver nitrate. From the data in table 2, it seems probable that the isolation could also be made by this means. The

TABLE 4.—*The separation of the potato ring-spot virus and the potato veinbanding virus by chemical treatment from a mixture of the viruses in plant extract*

Chemical	Conc. per cent	Number of infected plants out of five inoculated after treatment of virus mixture			
		with both viruses	with potato-veinbanding virus only	with potato ring-spot virus only	with neither virus
Mercuric chloride	0.1	5	0	0	0
	0.2	5	0	0	0
	0.3	0	5	0	0
	0.4	0	1	0	4
	0.5	0	0	0	5
Potassium permanganate	0.1	5	0	0	0
	0.2	5	0	0	0
	0.3	5	0	0	0
	0.4	5	0	0	0
	0.5	0	0	5	0
	0.6	0	0	5	0
Sodium hydroxide	0.1	5	0	0	0
	0.2	0	0	5	0
	0.3	0	0	0	5
Nitric acid	0.1	5	0	0	0
	0.2	0	0	5	0
	0.3	0	0	0	5
None (check)		5	0	0	0

results shown in table 4 have been repeated 3 times with almost identical results. In one experiment, the inoculum was taken from plants with "spot necrosis" symptoms, *i.e.*, infected by both viruses simultaneously and the isolation of both viruses was completed by chemical means. In some cases repeated transfers were made to other tobacco plants to check the purity of the isolates. In every instance tested, the viruses isolated were found to be free from the other virus of the complex.

One attempt has been made by this method to isolate each of the viruses from artificially infected rugose-mosaic potato plants. The results have been partly negative since the latent-mosaic virus only was isolated. However, since the untreated controls yielded only 1 plant in 5 infected with the veinbanding virus, it was concluded that for some reason the veinbanding virus was not present in sufficient concentration to make the trial reliable.

Cucumber-mosaic Virus and Tobacco Ring-spot Virus Mixture. The viruses of cucumber mosaic and tobacco ring-spot in combination have not been reported as associated in nature in the production of any diseases. They, however, form an ideal combination for a study in which the aim is to investigate the possibilities of the separation of viruses by chemical inactivation. The above viruses have quite similar points of thermal inactivation and tolerance to dilution, but are separable by aging, insect transmission, and the use of differential hosts. In combination, they do not produce such

striking symptoms as do the 2 previously studied complexes, each virus continuing to produce characteristic symptoms, so the presence of either is not masked. Generally, the plants are stunted more than when infected by either virus alone.

As shown in table 2, the cucumber-mosaic virus is slightly more tolerant to most chemicals than the tobacco ring-spot virus. Phenol and oxalic acid, however, appear to be less toxic to the latter virus than to that of cucumber mosaic. Consequently, the combination of the 2 viruses was treated with phenol to inactivate the cucumber-mosaic virus and with mercuric chloride to inactivate that of ring spot. The experiment was performed, using methods similar in every respect to those of the 2 foregoing experiments.

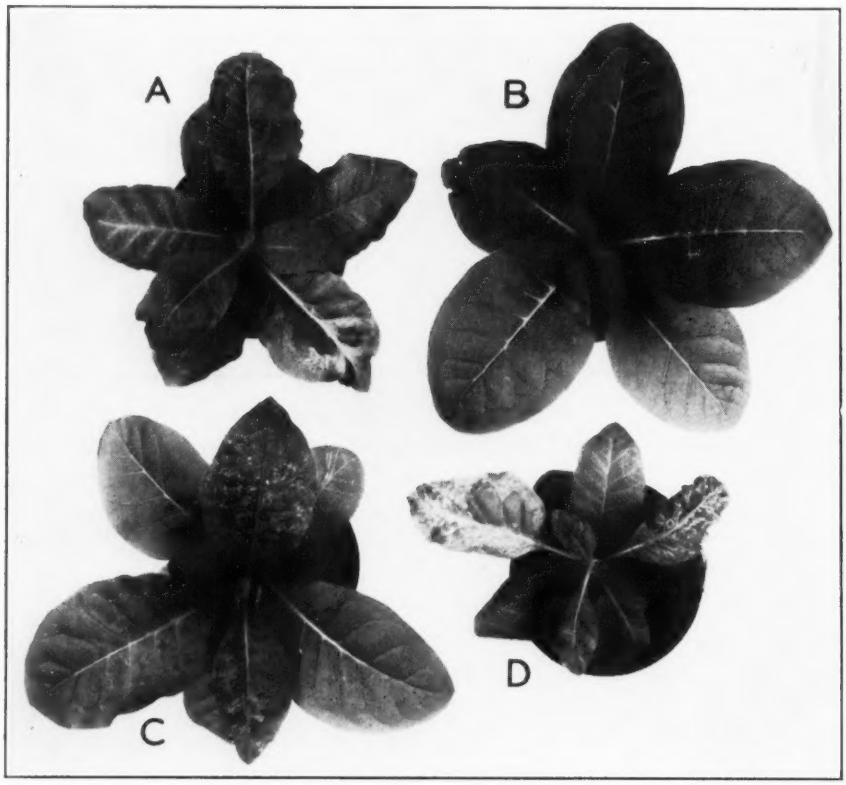

FIG. 2. The separation of the viruses of cucumber mosaic and tobacco ring spot. A. Plant inoculated with an extract containing both viruses, treated with 0.1 per cent mercuric chloride. Infection with cucumber-mosaic virus only has developed. B. Healthy, noninoculated control. C. Plant inoculated with the same mixture as A, but treated with 4.0 per cent phenol. Symptoms of tobacco ring spot only are evident. D. Plant inoculated with the nontreated extract containing both viruses.

The results of a typical experiment are given in table 5. As expected, the combination was resolved into its 2 component viruses (Fig. 2). In 4 trials, 4.0 per cent phenol completely inactivated the cucumber-mosaic virus, leaving the tobacco ring-spot virus infective. In a fifth trial, when 5 plants

TABLE 5.—*The separation of viruses of cucumber mosaic and tobacco ring spot by phenol and mercuric chloride from a mixture of the viruses in plant extract*

Chemical	Conc. per cent	Number of infected plants out of five inoculated after treatment of virus mixture			
		with both viruses	with cucumber-mosaic only	with tobacco-ring-spot only	with neither virus
Mercuric chloride	0.4	0	5	0	0
	0.5	0	5	0	0
	0.6	0	5	0	0
	0.7	0	5	0	0
	0.8	0	0	0	5
Phenol	1.0	5	0	0	0
	2.0	5	0	0	0
	3.0	5	0	0	0
	4.0	0	0	5	0
	5.0	0	0	0	5
None (check)		5	0	0	0

were inoculated with an extract treated with 4.0 per cent phenol, 3 plants were infected with tobacco ring spot only, 1 plant with cucumber mosaic only, and 1 with both viruses. In most instances, 5.0 per cent phenol completely inactivated both viruses when they were in combination. Occasionally, however, the tobacco ring-spot virus remained infective. This result with phenol does not agree perfectly with the results given in table 2, where 5.0 per cent phenol is shown not to completely inactivate either virus. The values given in table 2 may be slightly high because of the occasional failures of certain chemicals to completely inactivate at concentrations that ordinarily produce complete inactivation. In some cases, it is also very probable that the viruses in combinations do not attain their usual concentrations and for this reason may be less tolerant to the treatment given. The concentrations, for example, are reduced one-half at the outset, when the combinations are obtained, by mixing extracts from plants with both viruses.

Tobacco-mosaic Virus and Tobacco Ring-spot Virus Mixture. For many years investigators have concluded that the tobacco-mosaic virus is much more tolerant to chemicals than any other recognized plant virus. Consequently, the writer considered the isolation of other viruses from a mixture containing tobacco-mosaic virus by chemical inactivation as a challenge to be met in an attempt to prove the value of this method.

An examination of table 2 reveals the ordinary tobacco-mosaic virus to be much more tolerant than any of the other viruses to all the chemicals used, with the exception of phenol. Since the tobacco ring-spot virus appeared to be particularly tolerant to phenol, it was combined with tobacco mosaic virus in the ordinary manner of equal parts by volume of extract. Treatment with phenol and potassium permanganate in varying concentrations was then made, as in preceding experiments. Contrary to expectations, the tobacco-mosaic virus tolerated higher concentrations of both chemicals than did the

ring-spot virus. The concentration of a virus no doubt bears some relation to the amount of a chemical necessary to cause complete inactivation. On the basis of tolerance to dilution, the tobacco-mosaic virus is roughly 1000 times as concentrated as the tobacco ring-spot virus in ordinary fresh tobacco extract. Therefore, an effort was made to separate these viruses when more nearly in the same concentrations. An extract for treatment, therefore, was prepared that consisted of 1 part tobacco-mosaic extract and 99 parts tobacco ring-spot extract. The mosaic virus was nevertheless roughly 100 times more concentrated than the ring-spot virus, as may be observed from the results of dilution tests (Table 6). Employing the usual procedure, this mixture was treated with 0.5 per cent sodium permanganate and various concentrations of phenol.

The results of one experiment presented in table 6 indicate a complete sep-

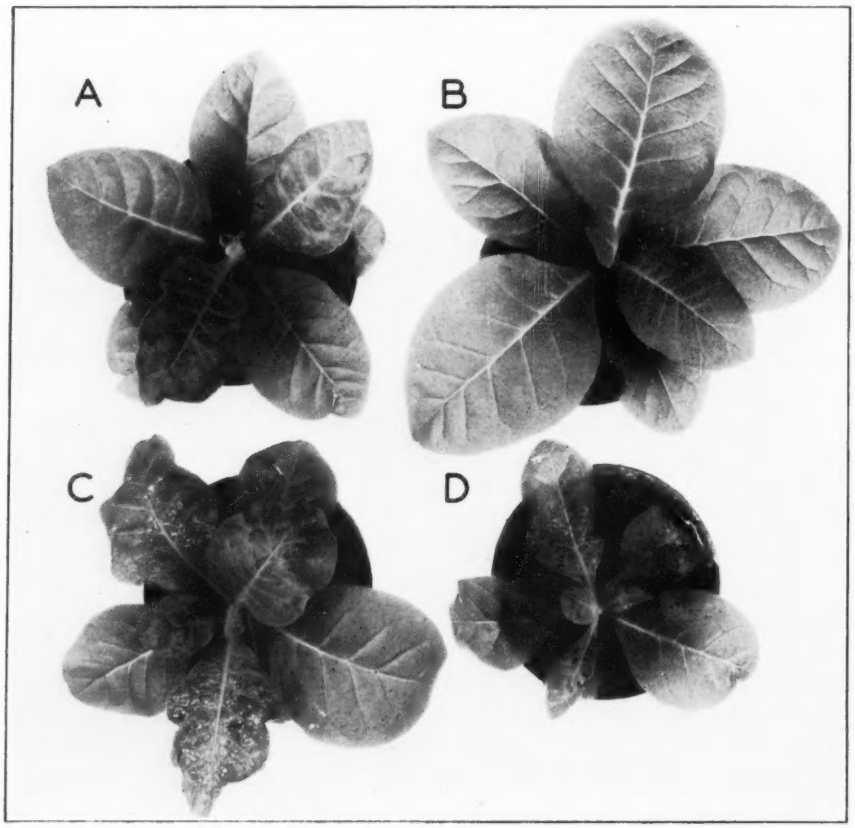

FIG. 3. The separation of the viruses of tobacco mosaic and tobacco ring spot with chemicals when the tobacco-mosaic virus has previously been diluted 1–100 (see page 904). A. Plant inoculated with an extract containing both viruses treated with 0.5 per cent sodium permanganate, showing the presence of tobacco mosaic only. B. Healthy, non-inoculated control. C. Plant inoculated with the same mixture as A, but treated with 4.0 per cent phenol. Note that tobacco ring-spot symptoms only are evident. D. Plant inoculated with nontreated extract containing both viruses. Symptoms of both virus diseases are present.

aration of the 2 viruses and, consequently, a specific action by the chemicals. Sodium permanganate inactivated the ring-spot virus without completely

TABLE 6.—*The separation of viruses of ordinary tobacco mosaic (diluted 1–100) and tobacco ring spot with phenol and sodium permanganate from a mixture of the viruses in plant extract, together with the effect of dilution upon the nontreated extract*

Chemical	Dilution	Conc. chem. per cent	Number of infected plants out of 5[a] inoculated following treatment of virus combination as shown			
			with both viruses	with tobacco-ring-spot virus only	with tobacco-mosaic virus only	with neither virus
		1.0	5	0	0	0
		2.0	5	0	0	0
Phenol		3.0	5	0	0	0
		4.0	0	5	0	0
		5.0	0	0	2	3
Sodium permanganate		0.5	0	0	5	0
	1–10		10	0	0	0
	1–100		8	0	2	0
Water	1–1000		0	0	10	0
	1–10,000		0	0	5	5
	1–100,000		0	0	2	8

[a] In case of water dilutions 10 plants were inoculated.

destroying the tobacco-mosaic virus, whereas 4.0 per cent phenol inactivated the tobacco-mosaic virus without completely destroying the ring-spot virus. The comparative symptoms are illustrated in figure 3, and a check made on the isolates by repeated inoculation to tobacco and the *N. glutinosa-N. tabacum* hybrid verified the purity of the respective viruses. This experiment has been performed 3 times with almost identical results in each trial. Concentrations of phenol below 4.0 per cent have not completely inactivated either virus. In one experiment, one plant of the group of 5 inoculated with the combined extracts treated with 4.0 per cent phenol was infected with tobacco-mosaic virus. Tobacco ring-spot symptoms occurred in only one plant of those inoculated with the extract containing 5.0 per cent phenol. Without exception, tobacco mosaic has appeared in from 1 to 3 plants in each set inoculated with the extracts treated with 5.0 per cent phenol. That is, 5.0 per cent phenol gave less inactivation of the tobacco-mosaic virus than 4.0 per cent phenol. The reason for this unusual result is obscure, although a possible explanation may be offered. When crude tobacco extract is treated with 5.0 per cent phenol, considerably more coagulation and clumping occurs than in the preparations treated with 4.0 per cent phenol. Since the tobacco-mosaic virus is roughly 100 hundred times more concentrated than the ring-spot virus in these extracts, it is not improbable that the chances for occlusion of the mosaic virus in the clumps is much greater. When the virus is surrounded by a mass of coagulated and precipitated material, it may not be readily accessible to the action of phenol.

Only 4 virus mixtures have been treated with chemicals; therefore, this work must be considered as of a preliminary nature. A sufficient number of repetitions, however, have been made to give assurance that at least in these virus mixtures separation by chemical inactivation is a reliable method.

DISCUSSION

The action of toxic substances has been widely studied in microbiology and the value of a large variety of chemicals for differential as well as for disinfection purposes is especially obvious. An attempt to utilize these principles in virus studies appeared logical, although at first obscure. The results secured from this study, nevertheless, are of considerable interest and suggest many possibilities for further investigation.

The repeated isolation of all the viruses in the 4 combinations studied indicates that separation by chemical specificity is feasible and may become a useful adjunct to the established methods of isolation. Satisfactory separation depends upon complete inactivation of all but one of the viruses present. Therefore, to be reliable, the specific behavior of chemicals must be great enough to offset factors such as variability in concentration of virus or in composition of plant extract, which might appreciably alter the tolerance of the virus to chemicals. Greater specificity probably would be found in purified virus preparations; but, until more viruses have been purified, a study of this type must be limited to the use of crude extracts.

The nature of the inactivating action and the specificity is obscure. Action of the chemical may be directly on the virus, indirect through chain reactions in the plant extracts, or possibly it may affect the susceptibility of the host to infection, as is claimed by Stanley (14) for the inactivating action of trypsin. In some cases the inactivation probably is reversible, as has been shown by Thornberry (16). Hydrogen-ion concentration seems not to be correlated with specificity, but the possibility is recognized that it might affect the toxicity of the different chemicals and thereby affect specificity. All the substances that were found to be good inactivators are protein denaturants and possess high germicidal properties. However, two potent germicides (Merthiolate and "mercury oxycyanide") were extremely weak inactivators.

It is highly desirable that future work concerning the identification and the separation of viruses by chemicals be conducted under more uniform conditions as to time and temperature of treatment. The lack of such uniformity in the past makes an adequate evaluation of previous work of this type difficult or impossible. A standard set of conditions should be adopted that falls within the working range of all the viruses. For example, a time of treatment as long as one day is generally unsatisfactory because many viruses naturally are inactivated by aging *in vitro* in that time. The writer has found the conditions used in the present experiments satisfactory, namely, 1-hour treatment at 20° C., of crude plant-virus extract with an equal volume of the chemical at double strength. It is suggested that this

set of conditions be tentatively adopted as a standard basis for future studies of a similar sort. One hour is less than the inactivation time by aging *in vitro* of practically all sap-transmissible viruses. The temperature of 20° C., is convenient to maintain in any laboratory. The final dilution (1–100) used in these studies may be high, but it is satisfactory for most viruses, and in some instances may be varied with little or no complication. The influence of host plants used as a source of the inoculum for chemical-inactivation studies may be in some doubt. Adequate data are not available to show what influence the host may have in such studies, although in a preliminary study Johnson and Grant (10) concluded that the viruses have somewhat characteristic tolerances to chemicals regardless of the host in which they are found. Since tobacco is commonly used in virus studies, probably the inoculum for treatment may best be obtained from this host whenever possible.

Many possibilities for future development of this problem are evident. For convenience of observation, only viruses giving necrotic and chlorotic types of symptoms have been used in this investigation. The writer, however, can conceive of no logical reason why all sap-transmissible viruses would not be amenable to this technique of separation. With this in mind, a study should be made to determine if chemicals of the same type have characteristic differential values, as is indicated by some results obtained in the present investigation. The greatest interest and value of studies of the type reported in this paper may lie in the eventual use of chemicals for the differentiation and separation of specific viruses not readily distinguished or isolated by other means.

The possible relation of chemical specificity to certain other problems concerning viruses is readily recognizable. The high degree of specificity for viruses noted in many insect vectors may possibly be related to this phenomenon. Chemotherapy sometimes depends almost entirely upon specific toxicity. Virus sanitation and prophylactic measures depend in some measure at least upon the specific action of chemicals. In plant-virus sanitation, the virus of ordinary tobacco mosaic is of special interest. Allard (1) reported complete inactivation of this virus with 4.0 per cent formaldehyde in 20 minutes. In the investigation here described, it was found that the strongest concentration of formalin (approx. 40.0 per cent formaldehyde) used (50.0 per cent) did not completely inactivate this virus in plant extract in 1 hour (Table 2). Other investigations have shown that Allard may have been working in part with the cucumber-mosaic virus, which at that time was not clearly differentiated from the tobacco-mosaic virus. The result obtained by Allard for the inactivation of tobacco-mosaic virus with formaldehyde agrees quite closely with the result obtained in this investigation with cucumber-mosaic virus (Table 2). These results show that the common practice of treating tobacco seedbed frames, etc., with formalin or mercuric chloride to destroy the tobacco-mosaic virus may be quite inadequate. Strong bases appear to be sufficiently toxic to warrant their trial in such sanitary measures. The application of chemical specificities to

virus problems is an open field from which many interesting and valuable developments may be expected.

SUMMARY

The inactivating effect of 35 chemicals upon the viruses of ordinary tobacco mosaic (*tobacco virus 1*), ordinary cucumber mosaic, potato ring-spot, potato veinbanding, tobacco ring-spot, and tobacco streak has been studied. The chemicals were selected on the basis of their known antiseptic properties and diverse mode of action as toxic agents.

The differential action of certain chemicals on the viruses used has been demonstrated. For example, the ordinary cucumber-mosaic virus is more tolerant to mercuric chloride and silver nitrate than is the potato ring-spot virus, but the cucumber-mosaic virus is less tolerant to copper sulphate, potassium permanganate, or lithium carbonate than is the potato ring-spot virus.

Each of the components of the following virus mixtures have been isolated repeatedly by treatment with specific chemicals: potato ring-spot virus plus ordinary cucumber-mosaic virus, potato ring-spot virus plus potato veinbanding virus, tobacco ring-spot virus plus ordinary cucumber-mosaic virus, and ordinary tobacco-mosaic virus plus tobacco ring-spot virus.

It is believed that the utilization of the differential action of chemicals on viruses may become of considerable value for purposes of their isolation and identification where other means are inconvenient or inadequate.

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OVERWINTERING OF TAPHRINA ROBINSONIANA[1]

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The generic limitations of *Exoascus* and *Taphrina* proposed by Sadebeck (6), based upon the presence or absence of perennial mycelium, have been accepted by many mycologists. The concept of Giesenhagen (2), merging all legitimate species of *Exoascus, Taphrina,* and *Magnusiella* in one genus, *Taphrina,* is accepted by the writer and by most workers of this generation. At present no noteworthy or practical characteristics have been discovered that justify the use of more than one genus for the species of this group of fungi.

It has been demonstrated by Mix (4) and by R. E. Fitzpatrick (1) that *Taphrina deformans* (Berk.) Tul. does not possess a perennial mycelium. Mix (3) has shown also that *T. mirabilis* (Atk.) Gies. depends upon overwintered spores for inoculum for initial infections. Both of these species overwinter, according to Sadebeck (6), by means of perennial mycelium, and were, therefore, included in the genus *Exoascus,* as understood by him.

Taphrina robinsoniana Gies. is the only species commonly affecting bracts of female catkins of *Alnus incana* (L.) Moench. in New York State (Fig. 1). For the past several years the disease has been very severe in central New York, and no trees of the great number observed have escaped some degree of infection. About mid-July the hypertrophied bracts make their appearance, and fresh material may be collected from that time until September. After the first deformed bracts appear, others retarded in their development by dry weather, appear during succeeding periods of rain.

Taphrina amentorum (Sad.) Rost., which occurs commonly in Europe, affects the bracts of the female catkins of *A. incana* and *A. glutinosa* Gaertn. just as does *T. robinsoniana.* This European species, according to Sadebeck (6), Giesenhagen (2), and others, is said to overwinter by means of perennial mycelium in the buds. Although the 2 species under discussion differ morphologically, the mycelium in both is subcuticular only and both species produce identical symptoms. Since they are similar in several respects, it might be expected that their life cycles are similar. No acceptable evidence has ever been presented to prove that *T. amentorum* actually possesses a perennial mycelium, though most discussions of it include a reference to its assumed perennial nature.

Giesenhagen (2), who named *Taphrina robinsoniana,* did not mention perennial mycelium, probably because he had only mature, dry material with which to work. Patterson (5), who confused the two species and

[1] A portion of a thesis presented to the faculty of the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer wishes to express his appreciation and thanks to Professor H. M. Fitzpatrick, who proposed the problem and offered helpful suggestions, and to Professor H. H. Whetzel for a critical reading of the manuscript.

FIG. 1. Female catkins of *Alnus incana* showing deformation of bracts caused by *Taphrina robinsoniana*.

called all of her collections *E. amentorum* Sad., stated that the mycelium is perennial, but gave no evidence to support her statement.

Experiments have been made by the writer in 2 successive years to determine whether *Taphrina robinsoniana* does overwinter by means of perennial mycelium, or whether spores instead, are not responsible for primary infections.

HISTOLOGIC EXAMINATIONS

In early April of 1936 a number of female catkins from trees that had been badly infected the season before were collected and brought into the laboratory for examination. Some of the aments were fixed, imbedded in paraffin, sectioned, and stained according to accepted methods of cytological technique. Others were cleared in lacto-phenol solution and stained with either cotton blue or acid fuchsin. Although a large number of catkins were examined, no mycelium was found. Later in the summer the trees from which specimens had been collected for these studies were found to be practically 100 per cent diseased. On the basis of histologic evidence, *Taphrina robinsoniana* does not possess a perennial mycelium.

SPRAYING EXPERIMENTS

The effect of spraying on the control of the disease was investigated. It could be assumed that spraying will give no control, if the fungus overwinters by means of a perennial mycelium.

A series of spraying experiments was carried out during the spring of 1937. On April 25, 6 young trees in a large group were selected and received a dormant spray of lime-sulphur solution, 1:40. Application was made with a quart-size hand sprayer. On May 2 another application was made on the same 6 trees. Two nearby trees were chosen as checks. On July 19, when the asci were formed and were discharging their spores, the sprayed and check trees were examined and estimates of the percentage of infection made. No attempt was made to count the diseased catkins, for it was obvious that the sprayed trees had fewer of them than the nonsprayed checks. The percentage of infection on the checks was between 90 and 100, while the estimated percentage of infection for the 6 sprayed trees averaged 19. Improved methods in the application of the spray should give better control. The results, however, indicate that overwintered spores induce primary infections.

BAGGING EXPERIMENTS

Another experiment was conducted in which clusters of female catkins were enclosed in transparent, water-proof bags. As many as 12 and not less than 3 female catkins in a cluster were sprayed with a 1:40 lime-sulphur solution, and over each of 25 clusters was placed a bag, the top of which was twisted tightly around the branch and made secure with a cord. In addition, 5 nonsprayed clusters of female catkins were enclosed by bags. These served as checks.

The experiment was begun in April, 1937, and the bags were not opened until July 19. At that date the catkins were examined. The catkins and leaves were well developed, green, and without apparent infection in any of the 30 bags. Exposed catkins on the bagged branches were in every case heavily infected. The bags were left off the catkin clusters until August 21, when an examination showed that every catkin remained healthy.

Why the nonsprayed catkins enclosed by the bags should remain healthy also is not easily answered, but it is the writer's belief that any spores that might have been on the surface of the host parts enclosed by bags failed to find conditions favorable for germination and penetration into the bracts. If the mycelium were perennial, its development within the host should have occurred, for the catkins and leaves developed normally and did not show any ill effects of having been enclosed by the bags.

The evidence presented by the writer, *i.e.*, failure to demonstrate perennial mycelium in the buds by histologic methods, lowering of the percentage of infection by application of a dormant spray, and absence of infection when the catkins were enclosed by bags, indicate that *Taphrina robinsoniana* does not overwinter by means of a perennial mycelium.

LONGEVITY OF ISOLATES

Several facts at hand suggest that spores of *Taphrina robinsoniana* could overwinter on the surfaces of the host. Examination of overwintered, diseased bracts in the spring disclosed spores in some of the asci.

Isolations of the fungus from bracts were made in January by means of the dilution-plate method. If spores remained viable until that time, with low temperatures prevailing until the inoculation period in the spring, it is logical to assume that the spores would still be capable of causing infection.

Pure cultures, isolated by allowing spores to shoot onto agar plates from freshly collected specimens, were streaked on potato-dextrose agar slants in July and held at 24° and 27° C., respectively. Transfers to fresh tubes of media were made at regular intervals to test viability. The cultures remained alive until January at 27° C. and until March at 24° C. At these times the periodic transferring was discontinued because the media in the original cultures dried out. The ability of the fungus to withstand these higher temperatures for such long periods of time indicates that the spores could survive in nature from September until the inoculation period at normal winter temperatures.

It is possible and very probable that the spores lying free upon the surface of the buds and twigs are able to multiply saprophytically. The ability to multiply in nature would make certain the retention of life of the spore for at least the overwintering period. Even if such a multiplication did not occur, it is reasonable to expect, on the basis of the data presented, that spore viability could be maintained over winter.

SUMMARY

Histologic studies of dormant female catkins of *Alnus incana* disclosed no perennial mycelium in the tissues.

Applications of 1:40 lime-sulphur solution, as a dormant spray, reduced the percentage of infection considerably.

Female catkin-clusters enclosed in transparent, water-proof bags reached maturity without becoming infected.

Results of cultural studies suggest that spores could remain viable in nature from the time of their dispersion until the inoculation period.

The writer concludes from his investigations that *Taphrina robinsoniana* does not overwinter by means of perennial mycelium in the tissue of bracts of female catkins of *Alnus incana*.

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STUDIES ON PEA VIRUS 1

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INTRODUCTION

Pea virus 1 differs from other viruses producing diseases in legumes in undergoing an incubation period of about 12 hours before it can be transmitted by the vectors *Macrosiphum pisi* Kaltenbach and *M. solanifolii* Ashmead (*M. gei* Koch) (3, 5). Although the virus is difficult to transmit by the ordinary rubbing method, transmission has been secured in a high percentage of the plants inoculated by following the method of Rawlins and Tompkins (7), using carborundum powder as an abrasive. Transmission by this method has made it possible to study some of the physical properties of the virus *in vitro*. In addition, studies have been made on the efficiency of mechanical transmission as compared with aphid transmission, on the effect of exposing viruliferous aphids to high temperatures, and on the occurrence of distinct strains of the virus. The purpose of this paper is to present the results of these studies.

EXPERIMENTS

Transmissibility of Pea Virus 1 from Plants Inoculated by Aphids and from Plants Inoculated Mechanically

Preliminary experiments indicated that pea virus 1 could be transmitted more readily from aphid-inoculated plants than from mechanically inoculated plants. In early trials virus from *Vicia faba* L. plants showing pronounced symptoms of disease about 3 weeks after inoculation by aphids was used. Of 60 plants inoculated by rubbing, 31 became diseased. Sub-inoculations were then made from these diseased plants after an equal period of time to determine whether the virus could be maintained mechanically by serial passage in *V. faba*. No infection was obtained in the plants sub-inoculated. No constant differences had been noticed in the symptoms of disease produced in the aphid-inoculated plants when compared with those inoculated mechanically, except perhaps a tendency for the symptoms to be more severe and to spread more rapidly in the aphid-inoculated plants.

To determine definitely whether first symptoms of disease appear later in plants inoculated mechanically than in plants inoculated with colonies of aphids, an experiment was conducted in which the inoculated plants were observed daily for first symptoms of disease. Two sets each containing 30 plants were inoculated. One set was inoculated by exposure for 2 days to pea aphids that had fed on diseased plants, 25 aphids being placed on each plant. The other set was inoculated mechanically with extracts from the same diseased plants on which the aphids had fed. Of the 30 plants inoculated by aphids, 29 became diseased, while of the mechanically inoculated plants, 19 became diseased. Of the 29 plants infected by aphids, first symp-

toms were observed in 4 to 5 days in 27 of the plants. First symptoms appeared on 1 plant on the 6th day and on 1 plant on the 7th day. Of the 19 plants infected by mechanical inoculation, 3 showed symptoms in 5 days, 6 in 6 days, 3 in 8 days, 2 in 9 days, 2 in 10 days, 1 in 12 days, 1 in 14 days, and 1 in 16 days. This experiment demonstrated that first symptoms of disease appear somewhat more promptly in aphid-inoculated plants than in mechanically inoculated plants.

In an effort to explain the difference in transmissibility, experiments were planned to compare transmission from aphid-inoculated plants with that from plants inoculated mechanically under conditions as nearly identical as possible. In one experiment pea aphids were fed for 2 days on 5 diseased *Vicia faba* plants that had been inoculated by aphids 75 days previously. The aphids were then removed and the juice extracted from the diseased plants. One set of 20 *V. faba* plants was then inoculated by exposure of each plant to 25 aphids for 2 days, while another set of 20 was inoculated mechanically with juice extracted from the diseased plants. Of the 20 plants exposed to aphids, all became diseased, while 14 of the 20 inoculated mechanically became diseased. Ten diseased plants were selected from each lot and were tested in pairs. The first test was made 17 days after inoculation of the plants. In tests with aphids a plant from each of the lots was placed in each of two adjacent cages in the greenhouse. About 150 pea aphids were fed on each of the diseased plants for from 24 to 48 hours to insure proper incubation of the virus in the insects. On removal of the aphids the plants were crushed and the juice from each was rubbed on the leaves of 5 small plants. Five small plants were also exposed for at least 24 hours to each lot of aphids removed from the diseased plants. This procedure was repeated for the tests with each pair of diseased plants at various intervals after inoculation. The results are shown in table 1. No

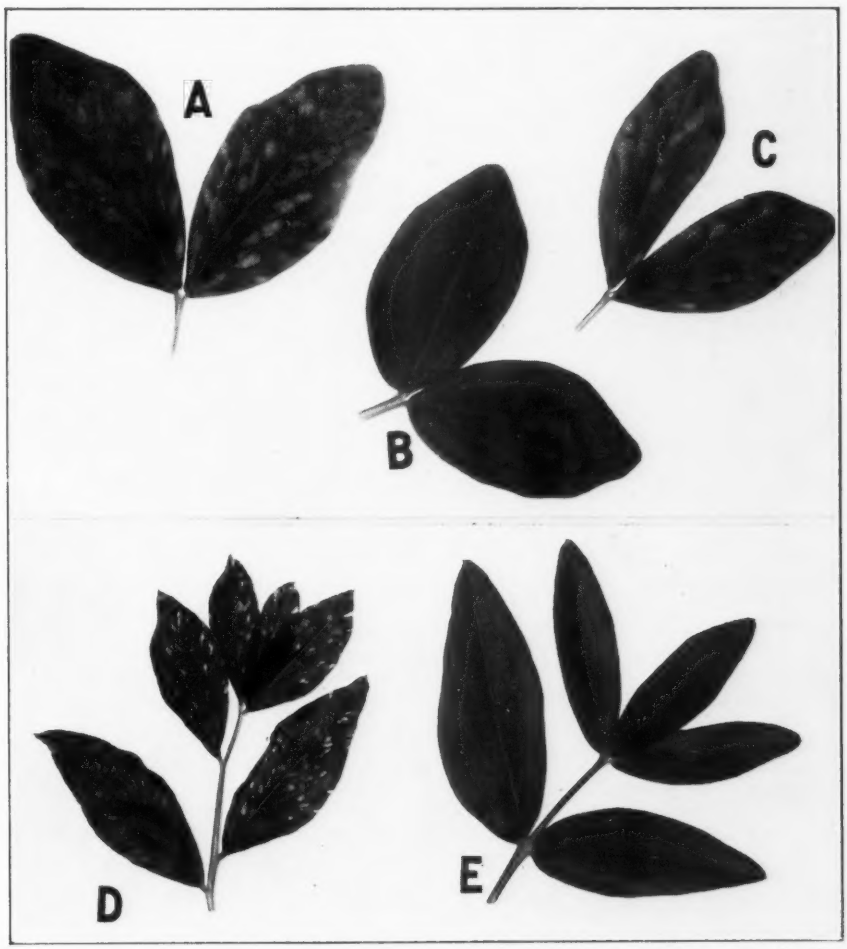
TABLE 1.—*Infectivity of juices from Vicia faba plants inoculated by insects as compared with juices from V. faba plants inoculated mechanically*

Test number	No. days after inoculation	Infections from insect-inoculated plants		Infections from mechanically inoculated plants	
		By insects	By rubbing	By insects	By rubbing
1	17	3[a]	5	0	0
2	22	5	2	0	0
3	26	5	5	0	0
4	30	5	5	0	0
5	33	5	5	0	2
6	35	5	5	0	0
7	36	5	5	0	0
8	40	2	5	0	2
9	42	5	5	0	2
10	45	5	5	3	5
		45	47	3	11

[a] Five *Vicia faba* plants were used in each test.

infection was obtained either by aphids or by mechanical inoculation from mechanically inoculated plants in the first 4 tests, while transmissions were obtained by both methods from the aphid-inoculated plants. In the other 6 tests much better transmission was obtained from insect-inoculated plants than from mechanically inoculated plants. Infection was obtained from the mechanically inoculated plants only in the case of those in which the virus had undergone an incubation of at least 33 days. The results suggest that increase of virus takes place much more slowly in mechanically inoculated plants than in insect-inoculated plants. The observation that frequently symptoms appear more slowly in mechanically inoculated plants than in aphid-inoculated plants strengthens this suggestion.

The difference in symptoms frequently observed in plants inoculated with virus by means of aphids and in plants inoculated mechanically is shown in



Photographs by J. A. Carlile

FIG. 1. *Vicia faba* leaves. A. From plant inoculated by means of pea aphids. B. From healthy plant. C. From plant inoculated mechanically. D. From plant infected with the strain of virus from New Jersey. E. From healthy plant.

figure 1, A and C. Leaves from 2 plants, inoculated at the same time, were photographed 16 days after inoculation.

Two of the diseased plants in this experiment that failed to give infection at the first test were retested at a later time. One mechanically inoculated plant that failed to give transmission when tested 17 days after inoculation was retested 52 days after inoculation. In the second test, no virus was recovered from the diseased plant by means of aphids, but 3 of 5 plants inoculated mechanically with juice extracted from the plant eventually developed mild symptoms of disease. Another mechanically inoculated plant that failed to yield virus when tested 22 days after inoculation was retested 62 days after inoculation. In the second test no virus was recovered by means of aphids, but, again, 3 of 5 plants inoculated mechanically slowly developed mild symptoms of disease, demonstrating that in some cases diseased plants that fail to yield virus in one test may yield virus at a later time.

The experiment was continued by using as a source of virus the diseased plants obtained by sub-inoculation. In some cases plants showing severe symptoms were selected for testing. In other cases plants showing faint symptoms were tested. The results presented in table 2 show that there is

TABLE 2.—*Infectivity of juices from Vicia faba plants inoculated by insects as compared with juices from V. faba plants inoculated mechanically*

Test number	No. days after inoculation	Infections from insect-inoculated plants		Infections from mechanically inoculated plants	
		By insects	By rubbing	By insects	By rubbing
1	34	0[a]	2	0	0
2	30	5	5	0	3
3	29	5	5	0	1
4	26	0	0	0	0
5	22	0	5	0	0
5a[b]	24	5	5	5	5
5b[c]	27	0	0	0	0
6	31	5	5	5	1
7	32	5	5	5	3
8n[d]	32	0	1	5	5
9	31	5	5	0	0
10	31	5	3	5	5
	Total	35	41	25	23

[a] Five *Vicia faba* plants were used in each test.
[b] 5a—Source plants that showed rapid spread of symptoms were selected.
[c] 5b—Source plants that showed slow spread of symptoms were selected.
[d] 8n—Source plants that showed slow spread and faint symptoms were selected.

considerable variability in transmission from aphid- as well as from mechanically inoculated plants. This test was made in the late fall and early winter under conditions that were not considered entirely satisfactory for uniform spread of the virus in the plants.

Since the experiments indicated that the length of the incubation period of the virus in the plants might be a factor influencing transmissibility, tests

were conducted with diseased plants that had been infected for short periods of time. Aphid-inoculated and mechanically inoculated plants that had been inoculated at the same time were tested simultaneously in adjoining cages in a greenhouse. In the tests for very short incubation periods, 50 aphids were placed on each inoculated plant for 1 day. Recovery of the virus was then determined by placing the aphids on 5 fresh *Vicia faba* plants and by crushing the inoculated plants and rubbing the juice into 5 fresh plants. The results are shown in table 3. The virus was not recovered from any plant

TABLE 3.—*Infectivity of juices from Vicia faba plants inoculated by insects as compared with juices from V. faba plants inoculated mechanically*

No. days after inoculation	Infections from insect-inoculated plants		Infections from mechanically inoculated plants	
	By insects	By rubbing	By insects	By rubbing
21	5[a]	2	0	0
14	5	5	0	0
8	2[b]	2[b]	0	2[b]
6	5	4		
4	2	2		
3	0	2[b]		
2	0	0		

[a] Five *Vicia faba* plants were used in each test.
[b] Faint or mild symptoms of disease produced.

within less than 3 days after inoculation. Virus was recovered by mechanical means from aphid-inoculated plants 3 days after inoculation and by means of aphids 4 days after inoculation. In one case very faint symptoms of disease were produced in 2 plants inoculated by rubbing with virus from mechanically inoculated plants tested 8 days after inoculation. After 14 and 21 days, respectively, virus was recovered from insect-inoculated plants but not from mechanically inoculated plants. These experiments demonstrated that virus is recoverable from aphid-inoculated plants 3 days after inoculation and at any time thereafter. They also showed that sub-inoculations from mechanically inoculated plants usually failed if made within 21 days after inoculation.

Serial Passage of Pea Virus 1 by Mechanical Methods

Experiments were made for the purpose of determining whether or not it would be possible to pass pea virus 1 in series mechanically. In one test a *Vicia faba* plant, severely infected 31 days after mechanical inoculation, was crushed and the juice rubbed into 5 fresh *V. faba* plants. Two of the 5 plants of the second passage became infected. After 130 days, leaves from these 2 plants were crushed and the juice rubbed again into 5 fresh *V. faba* plants. Two plants of the third passage showed mild infection. After 31 days, leaves from these 2 plants were crushed and rubbed into 5 fresh plants. One plant of the fourth passage developed very faint symptoms. Leaves from this plant were crushed after 38 days and rubbed into 5 fresh plants, but no symptoms of disease were produced.

In another test, 50 *Vicia faba* plants were inoculated with juice expressed from aphid-inoculated plants. Thirty-six became diseased. After 43 days, leaves from these plants were crushed and the composite sample of mixed juice was rubbed into 50 fresh plants. Eighteen plants of the second passage became diseased. Leaves from 16 of these were crushed 47 days later and the mixed juice from these was rubbed into 100 fresh plants. No symptoms developed in any of the plants of the third passage. When it became evident that no plants of the third passage were diseased as a result of mechanical inoculation, a test was made to determine if the virus could be recovered from the diseased plants of the second passage by means of aphids. For this test 3 diseased plants of the second passage were selected 60 days after inoculation. Aphids were fed on the plants for 3 days and then 75 were transferred to each of 10 small plants and allowed to feed for 3 days. No symptoms developed in any of the plants. These experiments demonstrate the difficulty of passing pea virus 1 in series mechanically.

Transmissibility from Plants Inoculated by Means of Single Aphids and from Plants Inoculated by Colonies of Aphids

Having observed that transmission was more certain and that symptoms appeared more quickly in plants inoculated by colonies of aphids than in plants inoculated by single individuals, it seemed of interest to test the transmissibility from such plants. The aphids used were large nymphs and adults that had been on diseased *Vicia faba* plants for 2 days. Colonies consisting of 30 individuals were placed on each of 20 *V. faba* plants and single aphids placed on each of another set of 20 plants. All of the plants exposed to colonies became diseased. Symptoms appeared in 4 to 5 days on 18 of the plants and in 6 to 7 days on the other two. Ten of the 20 plants exposed to single insects showed symptoms within 6 to 8 days and 3 others within 14 days. After 25 days, 5 plants from each lot were tested for transmissibility by both aphid and mechanical inoculation. Transmission was obtained in every instance from plants previously inoculated by colonies of aphids. Similarly, transmission was obtained in every instance by means of aphids from plants inoculated by single insects, but from only 2 of the 5 plants tested by means of mechanical inoculation. This experiment failed to indicate a significant difference in transmissibility from plants inoculated by colonies and those inoculated by single insects.

Properties of the Virus

Having found that pea virus 1 could be transmitted from aphid-inoculated plants by the carborundum powder method in a high percentage of trials, a series of experiments was undertaken to determine the physical properties of the virus. For this purpose diseased plants were crushed 4 to 8 weeks after inoculation by colonies of aphids, and the plant extract, after treatment, was rubbed into the leaves of small *Vicia faba* plants dusted with carborundum powder No. 320.

Thermal Inactivation. Resistance to heat was determined by placing 2 cc. of undiluted extract from diseased *Vicia faba* plants in thin test tubes, heating in a water bath at the desired temperature for 10 minutes, and then testing for infectivity by mechanical inoculation to *V. faba* plants. The results of several tests are summarized in table 4. Infection was obtained from juice

TABLE 4.—*Infections obtained by inoculation with virus exposed to different temperatures for 10 minutes*

Temperature (degrees C.)	No. plants inoculated	No. plants infected
Control	40	28
52	10	5
54	10	2
56	10	1
58	10	2
60	20	2
62	10	1
64	20	1
66	20	0

heated to various temperatures up to 64° C., but was not obtained from juice heated to 66° C.

Resistance to Aging in vitro. To determine resistance to aging *in vitro,* test tubes, each containing 2 cc. of expressed juice from diseased plants, were held at room temperature. After different intervals of time juice from the tubes was used for inoculation of *Vicia faba* plants. The results of these tests are shown in table 5. The virus was infective after aging *in vitro* for periods

TABLE 5.—*Infections obtained by inoculation with virus aged in vitro for different periods of time*

Time aged	Plants inoculated	Plants infected
2–6 hours	25	14
1 day	5	2
2 days	5	3
3 days	5	1
4 days	15	5[a]
5 days	15	0
7 days	5	0

[a] Symptoms slow to appear and of a mild type.

up to and including 4 days but was not infective after 5 days' aging. The appearance of symptoms in plants inoculated with virus that had aged for 4 days was delayed for a much longer period than in plants inoculated with juice that had aged for shorter periods.

Tolerance to Dilution. For determining tolerance to dilution, juice from diseased plants was diluted to the desired point with distilled water and then tested for infectivity by mechanical inoculation to *Vicia faba* plants. Since the thermal inactivation point and degree of resistance to aging *in vitro* found for pea virus 1 is very close to that previously shown for pea virus 2 (4), it seemed of interest to compare the tolerance to dilution by these viruses under

comparable conditions. The results are shown in table 6. Pea virus 1 was infective at a dilution of 10^{-3}, but was not infective at a dilution of 10^{-4}. Pea

TABLE 6.—*Infections obtained by inoculation with virus after various degrees of dilution*

Dilution	Pea virus 1		Pea virus 2	
	No. plants inoculated	No. plants infected	No. plants inoculated	No. plants infected
10^{-0}	50	36	20	18
10^{-1}	50	19	20	17
10^{-2}	50	12	20	17
10^{-3}	50	2	20	12
10^{-4}	50	0	20	4
10^{-5}	50	0	20	1

virus 2 was infective at a dilution of 10^{-5}. Dilutions greater than 10^{-5} were not tried.

Effect of Temperature on the Virus in the Aphids

Kunkel (2) has shown that the vector, *Macrosteles divisus* Uhl. (*Cicadula sexnotata* Fall.), always lost the ability to transmit aster yellows when held at a temperature of 32° C. for 1 day. The ability was regained on return to 24° C. Exposure for 12 days or more to a temperature of 32° C. permanently inactivated the virus without affecting the leaf hoppers. Since pea virus 1 is presumed to be held in the aphid vector in a manner similar to that in which aster-yellows virus is held in the leaf hopper, it seemed of interest to determine the effect of heat on pea virus 1 in the pea aphid.

In one experiment pea aphids were fed for 2 days on diseased *Vicia faba* plants in a cage in a greenhouse held at a temperature of 23° to 26° C. Seventy-five were then placed on each of 6 small *V. faba* plants in lamp chimneys in a room held at a constant temperature of 35° C. An equal number were placed on each of 6 small plants in the greenhouse. Both lots were transferred to fresh plants every day for 12 days. One lot was held in the hot room for 6 days and then in the greenhouse for 6 days, while the other lot was held in the greenhouse for the entire period. Of the 144 plants exposed, all became infected, demonstrating that the virus was still active in aphids that were held for 6 days at a temperature of 35° C.

In another test aphids that had fed on diseased plants were still infective after being held for 6 days at 35° C. on insusceptible red-clover plants.

In other experiments, requiring longer periods at 35° C., some of the colonies lost the ability to infect in 5 to 9 days and none were infective after periods longer than 9 days in the hot room. This loss of infectivity, however, may have been due to loss of infective individuals in the colonies. Mortality was higher than in the control colonies, and, after about 3 days in the hot room, the aphids no longer gave birth to normal nymphs. In one experiment the aphids appeared weak and were greatly reduced in number at the end of 8 days in the hot room. After 11 days, the colonies were reduced from

75 to an average of 5 individuals, whereas there was an average of 40 in the control colonies.

HOSTS

Broad bean (*Vicia faba*), garden pea (*Pisum sativum* L.), field pea (*P. sativum* var. *arvense* Poir.), sweet pea (*Lathyrus odoratus* L.) and crimson clover (*Trifolium incarnatum* L.) were reported in a previous paper to be susceptible to pea virus 1 (3).

Attempts to infect the following plants either by mechanical inoculation or by means of pea or potato aphids failed: red clover (*Trifolium pratense* L.), alsike clover (*T. hybridum* L.), white dutch clover (*T. repens* L.), white sweet clover (*Melilotus alba* Desr.), yellow sweet clover (*M. officinalis* (L.) Lam.) and alfalfa (*Medicago sativa* L.). Attempts to infect peanut (*Arachis hypogaea* L.) with the pea aphid, and tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum tuberosum* L.) with the potato aphid were likewise unsuccessful. Sub-inoculations were made from each of these plants to *Vicia faba*, but in no case was the virus recovered. The Green Stringless Refugee and Corbett Refugee varieties of the garden bean (*Phaseolus vulgaris* L.) also failed to become infected when inoculated by means of the pea aphid. No infection was produced by mechanical inoculation to any of the following plants: Turkish tobacco (*Nicotiana tabacum* L.), *N. glutinosa* L., *N. sylvestris* Spegaz. and Comes, *N. langsdorffii* Weinm., Green Stringless Refugee, Corbett Refugee and Robust varieties of the garden bean and mung bean (*P. aureus* Roxb.).

STRAINS OF PEA VIRUS 1

The virus used in the experiments reported here was the same strain of pea virus 1 that was employed in studies on transmission by the pea aphid (3). It was obtained originally from Westchester County, New York, and had been maintained in a greenhouse for a period of 6 years by serial passage in *Vicia faba*, using the pea or potato aphids as vectors. Another strain of pea virus 1 was obtained originally from pea aphids collected on pea plants growing near Princeton, New Jersey, and has been maintained in a similar manner for a period of 4 years by serial passage in *V. faba*.

The strain of virus from New York has already been described (3). The strain from New Jersey is essentially similar to the strain from New York in its transmission by aphids and in host range. It also produces the characteristic enations on the under surface of the leaves of crimson clover and peas and sometimes produces inconspicuous enations on the under surface of *Vicia faba* leaves. It differs slightly, however, in symptoms produced on *V. faba* and crimson clover and also in ease of mechanical inoculation. The strain from New York often produces large yellow spots on leaves of infected *V. faba* plants during early stages of invasion by the virus and sometimes these symptoms appear in the later stages as well. Figure 1, A and C, shows the large yellow spots frequently produced in *V. faba* plants diseased with the strain of virus from New York. In the first stages of invasion of young

V. faba plants the strain of virus from New Jersey often produces crinkling, distortion, and necrotic spots and splotches on the leaves. In later stages of invasion it is often difficult to distinguish the symptoms produced by the two virus strains. Figure 1, D, illustrates the spotted type of symptoms produced by the New Jersey strain of the virus in mature plants. When inoculated into crimson clover by aphids, early symptoms of the strain from New York consist of yellowish spots on the leaves, while, with the strain of virus from New Jersey, there is a tendency for the spots to be necrotic. No distinct differences have been observed in the symptoms produced by the two strains in garden-pea plants.

The strain of virus obtained in New Jersey has proved to be more difficult to transmit by mechanical inoculation in *Vicia faba* than the strain from New York. In some early experiments only a few infections were obtained. In a recent experiment, 50 plants were mechanically inoculated with juice expressed from plants diseased with the strain of virus from New Jersey and another 50 plants with juice extracted from plants diseased with the strain of virus from New York. Nine plants inoculated with the strain from New Jersey became infected, while 36 of the plants inoculated with the strain from New York became infected. It was because of this difference in degree of infectivity that experiments to determine properties of the virus *in vitro* were carried on with the strain of virus originally obtained in New York.

DISCUSSION

Previous papers on pea virus 1 (3, 5) have dealt largely with transmission by means of the pea and potato aphids. This was due to the fact that the virus is difficult to transmit by ordinary mechanical methods. In this respect it is similar to other viruses that require an incubation period in an insect vector. It has been demonstrated that pea virus 1 is readily transmitted by the carborundum-powder method. The results obtained, however, are quite different from those obtained with pea virus 2 (4), which is easily transmitted by ordinary rubbing methods and by aphids without the necessity of an incubation period in the vector. No difference in transmissibility of pea virus 2 has been observed between plants inoculated by aphids and those inoculated mechanically, and this virus is readily maintained by mechanical inoculation in serial passage in *Vicia faba*. On the other hand, there is a distinct difference in transmissibility of pea virus 1 from plants inoculated by colonies of aphids and from those inoculated mechanically. Sub-inoculations from mechanically inoculated plants have been more difficult to obtain and the virus is, therefore, not readily maintained in serial passage by mechanical transmission.

In plants inoculated mechanically the symptoms usually are milder and appear later than in plants inoculated by aphids. Apparently the virus spreads more slowly. These are symptoms often associated with "attenuation" of a virus. Whether or not the difference in transmissibility is due to

an "attenuation" of the virus is hard to say, since it is difficult to maintain the virus from these plants by serial passage in *Vicia faba*.

It is suggested that the difference in transmissibility may be attributable to the relative concentration of the virus in the plants. The virus may be present in a plant in sufficient concentration to produce symptoms and still not be present in sufficient concentration for transmission either by aphids or mechanically. It is possible that inoculations made by aphids fed on thoroughly diseased plants result in the virus reaching cells or tissues in which it multiplies and from which it spreads rapidly, whereas the virus inoculated by mechanical methods may often be localized in cells or tissues from which it spreads slowly. Occasionally, however, in a mechanically inoculated plant, symptoms appear promptly and are indistinguishable from those in the most thoroughly diseased plants inoculated by aphids. There seems to be no evidence, therefore, that the virus is changed by passage through the aphids.

It has been demonstrated that colonies of the pea aphid are more effective for the purpose of inoculating *Vicia faba* plants than is the carborundum-powder method. It should be emphasized, however, that it is necessary for the aphids to feed on thoroughly diseased *V. faba* plants in order to acquire the virus in sufficient amount for transmission to healthy plants. In fact, in some cases colonies of aphids have failed to acquire or transmit the virus from diseased plants from which it was recovered mechanically.

It has been difficult to transmit the virus from some aphid-inoculated plants. Whether this was because of the manner in which the plants were inoculated by aphids or whether it was because of the concentration of virus in the source plants from which it was obtained by the aphids is not known. The aphid-inoculated plants from which it has been difficult to transmit the virus have shown a mild type of symptoms. Plants inoculated by aphids after a short period of incubation of the virus in the aphids have sometimes produced very mild symptoms that were slow to develop. Similar results were sometimes obtained when inoculations were made by single insects. One test, however, failed to indicate a significant difference between transmissibility from plants inoculated by colonies of aphids and from plants inoculated by single aphids.

Snyder (8), Stubbs (9), Pierce (6), and Johnson and Jones (1) have reported on viruses in peas that produce the enations characteristic of pea virus 1. While these workers have not determined the mode of transmission by aphids, it seems quite evident that they were dealing with a virus similar to if not identical with pea virus 1. Stubbs suggested the name Enation Pea Mosaic for the disease and pea virus 1 for the virus producing it. Stubbs also pointed out that the Perfection variety of garden pea served to separate pea virus 1 from pea virus 2. The Perfection variety of pea is susceptible to pea virus 1 but is insusceptible to pea virus 2. The physical properties reported here for one strain of the virus agree fairly closely with those reported by Stubbs and Pierce. Johnson and Jones, however, appear to have been working with a strain more difficult to transmit by mechanical methods.

SUMMARY

Although pea virus 1 is difficult to transmit by the ordinary rubbing methods, it is transmissible by the carborundum-powder method.

Sub-inoculation from mechanically inoculated plants was found to be more difficult than from plants inoculated by colonies of aphids.

The virus was recovered from infected plants as soon as 3 days after inoculation by colonies of pea aphids. Sub-inoculation from mechanically inoculated plants usually failed if made within less than 24 days after inoculation, and in many plants after much longer periods of time. Sub-inoculation from aphid-inoculated plants failed in some cases.

The virus was carried through 4 serial passages by mechanical inoculation in *Vicia faba*. Sub-inoculation appeared to be even more difficult after several serial passages by mechanical inoculation in *V. faba*.

The virus was infective after heating *in vitro* for 10 minutes at various temperatures up to 64° C., but was not infective after heating to 66° C. It was infective after aging *in vitro* for periods up to 4 days, but was not infective after 5 days' aging. The virus was infective after dilution to 10^{-3}, but was not infective after dilution to 10^{-4}.

Aphids retained the virus for periods up to 8 days when they were removed from diseased plants and transferred to a succession of healthy plants that were held in a room at a temperature of 35° C. Failure of aphids to infect plants after being held for periods longer than 8 days at 35° C. may have been due to the loss of infective individuals in the colonies when held at this temperature.

Two strains of pea virus 1 are described. These differ in symptoms and in ease of transmission by mechanical means.

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A BACTERIAL BLIGHT OF STOCKS CAUSED BY PHYTOMONAS SYRINGAE

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(Accepted for publication Aug. 15, 1938)

In 1912 Briosi and Pavarino (2) reported on a disease of the flowering stock, *Matthiola incana* R. Br. var. *annua* Voss, and attributed the cause to a bacterium, which they named *Bacterium matthiolae*, n. sp. In a foot note they stated that the work would be published in the *Atti dell' Istituto Botanico dell' Universita di Pavia*. This second article (3) which was practically identical with the first, evidently appeared the same year in pamphlet form, since references have been found to it bearing the date 1912. It did not appear, however, in a volume of the above periodical until 1918. E. F. Smith (8), in reviewing the two publications, gave practically a complete translation. Rudorf and Job (7) reported the same disease in Argentina in 1932. This latter article has not been seen by the writer, but an abstract of it in the Review of Applied Mycology does not indicate that these investigators had the pathogen in culture. In 1934 Adams and Pugsley (1) reported a bacterial disease of flowering stock in Australia, and, while they described the pathogen, they declined to give it a name.

Both the Italian and the Australian organisms are green fluorescent bacteria, and, as far as described, they vary from *Phytomonas syringae* (van Hall) Bergey *et al.* in one character each. The Briosi and Pavarino pathogen was described presumably as gram-positive. E. F. Smith, translating literally from the Italian, states that "it resists Gram completely." It is not likely, however, that a green fluorescent bacterium is gram-positive. A few have been described but they have never been checked and proved correct. Bergey considers that his genus *Pseudomonas*, which contains the green fluorescent species, is a gram-negative genus. It is very easy to make a mistake in staining. The Adams and Pugsley pathogen is gram-negative, but it hydrolyzes starch. This might be correct, but the writer has worked with approximately 30 species of the green-fluorescent group, some of which have been reported to hydrolyze starch, and has found none that did. Maltose also was never utilized, although one would expect this if the starch were hydrolyzed. When the starch-agar-iodine method is used one should be careful to remove the colonies to be tested from the surface of a stiff agar. Otherwise, the iodine may not penetrate under the colonies and a light, spurious zone remains on the plate.

Since both these descriptions correspond so closely to that of *Phytomonas syringae*, the writer undertook to determine whether *Phyt. syringae* produces a disease of stocks and, if so, whether it resembles the disease described by these authors. Two isolates of this organism were on hand, one isolated from lilacs during the summer of 1937 and one from Lima beans isolated in November, 1937. Both were virulent cultures. When these isolates were inoculated

into flowering stock in the greenhouse a disease was produced similar to the ones described by Briosi and Pavarino and by Adams and Pugsley. The colored plates of Briosi and Pavarino assist in arriving at such a conclusion. Both strains of the pathogen were reisolated from the diseased tissues they produced. At the same time stocks also were inoculated with *Phyt. maculicola* (McCulloch) Bergey *et al.*, a bacterium similar in appearance and in biochemical reactions to *Phyt. syringae*. This pathogen was isolated July, 1937, from cauliflower, where it was causing a peppery spot of the leaves. No infection was obtained on stocks with the organism, although the cross inoculation was from one crucifer to another. The culture was virulent, since it produced infection both on cauliflower and on brussels sprouts at that time.

It is well known that *Phytomonas syringae* has a wide host range, but probably a definite one. That the stock is a host, we are certain from these experiments. The description of *Phyt. matthiolae* is so similar to *Phyt. syringae* that the two might be identical. The diseases they produce are similar. The writer, therefore, believes that *Phyt. matthiolae* is a synonym of *Phyt. syringae*.

The above bacterial disease of stock should not be confused with that reported by von Faber (5) and by Cooley (4) and attributed by them to *Phytomonas campestris* (Pammel) Bergey *et al.* Recently, this latter disease has been shown by Kendrick (6) to be caused by a pathogen similar in appearances to *Phyt. campestris*, but distinct. As yet, he has not named the organism.

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PHYTOPATHOLOGICAL NOTES

The Spread of Apple Mosaic.—In 1923 and 1924 it was reported[1, 2] that mosaic or variegation of apple, *Pyrus malus,* is transmissible by budding or grafting. Bradford and Joley[3] confirmed this finding, adding many observations on varietal behavior, and, further, uncovered the fact that this mosaic and its transmission by budding was reported in European literature early in the 19th century.

Following the transmission of this mosaic by budding and grafting in 1923, the author made various unsuccessful attempts to transmit the disease with various insects, mainly aphids and leaf hoppers. In the meantime many reports[4] had been received of its occurrence in various counties of New York State as well as from other States.

Since the preliminary attempts at transmission by insects had not given positive results, it seemed desirable to determine by survey methods whether there is any measurable natural spread. With this aim in view, a survey was made in 1927 of some of the orchards affected, followed by resurveys of the same orchards in 1932 and in 1937. Each tree in the orchards in question was examined and maps were prepared showing which trees were affected at each of these dates.

Many of these orchards are old and have suffered severely from winter injury, so that it is doubtful how many will last for another 5-year period. Also, the results to date are fairly conclusive, so that a brief report now seems desirable.

In the nine orchards surveyed in 1927, still extant in 1932, a total of 1207 trees was examined, of which 72 showed symptoms of mosaic in 1927 and 109 in 1932, thus making a 51.4 per cent increase for the 5-year period. At the end of the second 5-year period, a total of 981 trees was examined of which 96 showed mosaic in 1932, while at the end of the period, in 1937, there were 163 mosaic, an increase of 69.8 per cent. Since 3 orchards were removed and 1 added in the second period, not all these records extend the 10-year period; but, of 914 trees examined in both 1927 and 1937, there were 31 showing mosaic at the beginning and 138 at the end of the 10-year period. When examined by the X^2 test, all of these differences appear significant with odds higher than 100 to 1, the figures for the 10-year period being most highly significant.

In general, trees that once became affected remained so throughout

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[4] The first report and specimens were received from George Smith, special fruit disease agent in Orleans County, who believed spread was occurring in orchards under his observation. Numerous reports also were received from A. B. Burrell and W. D. Mills and several county agents.

this period, although there were very few foubtful cases that might have been due to errors in diagnosis or in mapping.

There are some indications, however, that this rather slow spread, should not be regarded as natural, *i.e.*, brought about through the agency of some insect vector, quite aside from the slowness of the spread. In several orchards, the trees recorded as mosaic at the time of the first observation, were largely in a single row, and subsequent spread had a tendency to follow the rows and in at least 2 cases in the direction followed in pruning operations.

Thus it appears certain that there has been a slow spread of the mosaic or variegation, but it appears probable that an explanation of this will be found connected with pruning operations rather than with insect transmission.—F. M. BLODGETT, Cornell University, Ithaca, N. Y.

An Apple Leafspot Associated with Fabraea maculata.—The Fabraea leaf blight of pear and quince is a common disease and is well known under a wide variety of environmental conditions in most parts of the world where these fruits are grown.[1] The writers are not aware, however, of any publication that describes the same organisms as causing a similar disease of apple. Massee,[2] in his general text on plant diseases, describes the organism as causing a leaf scald of "pear, apple, peach, quince, cherry, and other rosaceous fruit trees." Massee quotes the publications of Léveillé,[3] Sorauer,[4] and Galloway[5] as verification of his statement. In none of these is the disease described on apple, peach, or cherry.

During June, 1938, a severe case of leaf spotting of pear seedlings, French crabapple seedlings, and McIntosh apple scions, budded on various rootstocks, was observed in nursery rows at the U. S. Horticultural Station near Beltsville, Maryland. These observations led to the discovery that a great many of the lesions on the pear and the French crab leaves, and fewer on the McIntosh apple leaves, were bearing black, blisterlike acervuli typical of those caused by *Fabraea maculata* (Lév.) Atk. Microscopic examination revealed the presence of the typical *Entomosporium* conidia on the lesions on all the hosts. We have collected abundant specimens of the disease on the apple varieties and are prepared to distribute samples to investigators on request. —M. C. GOLDSWORTHY and M. A. SMITH, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

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PHYTOPATHOLOGICAL NOTES

Danger in Unguarded Seed Importation.—World agriculture seems as yet hardly to comprehend the extent to which the seeds of plants serve as potential carriers of destructive plant pathogens prevalent in different regions of the earth. In spite of Orton's excellent bulletin on seed-borne parasites[1] and other efforts to place before the public the growing evidence of the seed-dissemination of a wide range of plant diseases, there still appears to be very little public recognition of the fact that unguarded transportation of seeds from one continent to another is fraught with the danger of introducing previously absent disease-producing agents or biotypes into new regions. Moreover, there seems to be but slight general appreciation of the fact that the long and intensive work by plant breeders to build up superior varieties of crop plants resistant to the major diseases prevalent in their respective countries is constantly threatened with failure as long as there is danger of the seed-introduction of new parasites to which such superior varieties may be susceptible.

The importance of these considerations is emphasized by such announcements as that in July, 1938,[2] of a new anthracnose (*Colletotrichum* sp.) of cotton found in Manchukuo. No one knows the possible damage that might result were this introduced into America, but when it is considered that the ordinary cotton anthracnose (*C. gossypii*), seed-carried, was the predominant cause of destructive seedling blight in 9 important cotton States in 1938,[3] it at once becomes evident that a new cotton anthracnose accidentally introduced in seed might well have serious consequences like those confronting American mint growers from the spread of mint anthracnose[4] or American potato growers from the spread of the destructive bacterial tuber ring rot and wilt[5] both of which perhaps have been introduced unwittingly in propagating stock from some other continent.

It is a matter of satisfaction that a subcommittee of The American Phytopathological Society,[6] headed by M. T. Munn of the New York State Experiment Station, Geneva, is giving serious consideration to the problem of dealing more effectively with seed-borne plant diseases. This committee deserves active support in its constructive endeavors.—HOWARD P. BARSS, Washington, D. C.

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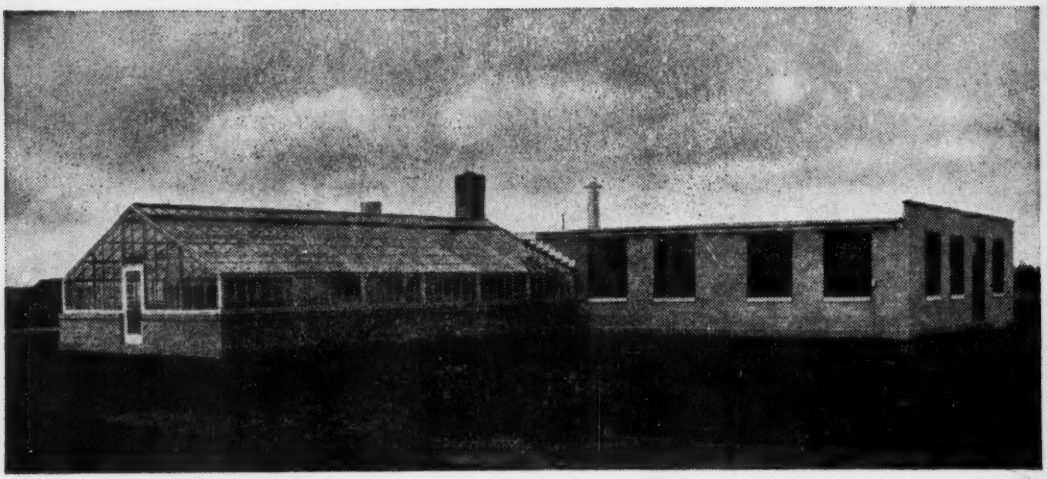

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